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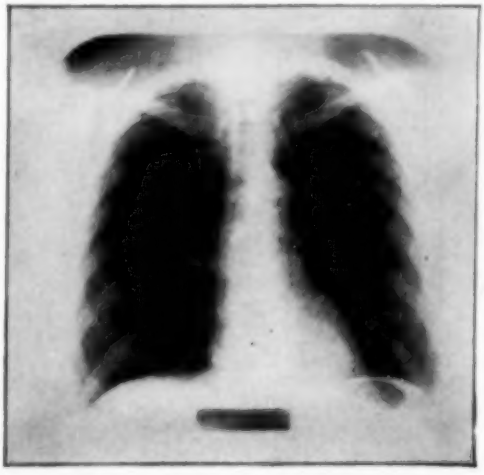

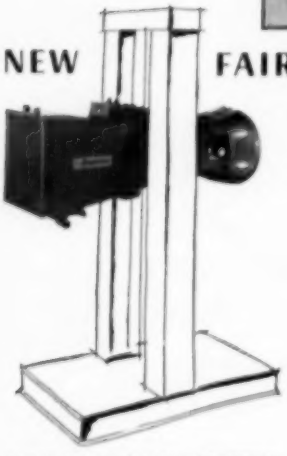

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New members of the Board as of July 1, 1955, are Dr. Charles A. LeMaistre, Dr. Richard L. Riley, Dr. David D. Rutstein, and Dr. Robert Shaw, who have accepted three-year appointments.

THE AMERICAN REVIEW OF
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A SCHEMA OF TREATMENT IN TUBERCULOSIS[1, 2]

CARL MUSCHENHEIM

(Received for publication April 22, 1955)

INTRODUCTION

The advances of the last ten years in the treatment of tuberculosis have revolutionized the management of this disease in all of its clinical forms. For all of us who have been much concerned with tuberculosis this has been an exciting, and sometimes even a breath-taking, adventure. Each new development has followed on another so rapidly that it has been quite impossible fully to evaluate a method of treatment before it is displaced by some modification or a totally new treatment which looks better. It is small wonder, therefore, that in this disease which has so many special characteristics, and for which the perfect treatment is not yet at hand, there should be now a period of uncertainty, confusion, and disagreement.

This uncertainty pertains not only to the selection of specific drugs and the indications for various surgical procedures, but even to the place where patients should be treated (in hospitals or at home) and by whom they should be treated. One group may wish to issue statements urging that all tuberculous patients be treated in tuberculosis hospitals, while another may suggest that special tuberculosis hospitals are rapidly becoming obsolete. There is not even agreement on the trends of prevalence, in this country and abroad, and no one seems to know whether the disease is becoming more or less important.

One thing, at least, we do know. Tuberculosis is still with us. Many patients with tuberculosis in one form or another appear in doctors' offices, in clinics, in hospitals, or are detected in surveys. They all require medical management, and many require active treatment. We cannot wait for the results of long-term follow-up studies now in progress, which may and probably will determine which of the currently available methods is the best in each type and variety of case. By the time these answers are forthcoming, more likely than not there will be new developments which will cause the best of present methods to be outmoded.

[1] From the Department of Medicine, New York Hospital-Cornell Medical Center, New York, New York.

[2] Address presented before the annual meeting of the North Carolina Trudeau Society, held at Durham, North Carolina, on April 13, 1955.

In the meanwhile there is the necessity to be systematic, not only in the comparative study of different modes of treatment, but in thinking about treatment problems. It is not enough that such excellent comparative studies be made as those organized by the Veterans Administration, the U. S. Public Health Service, and Great Britain's Medical Research Council. Not all patients can or should be treated according to chance selection. Indeed, control studies of this type have many limitations. Moreover, they are justified only when expected differences in results are relatively small and undeterminable by less arbitrary methods of clinical evaluation.

With full awareness of the present unpopularity of this viewpoint, I believe that too much emphasis is often placed on mathematically significant differences, when the basic observations are as subjective as, for instance, the roentgenographic evaluation of the progress of pulmonary tuberculosis under therapy. The very terms "better" and "worse" are incapable of precise definition in roentgenographic terms. When one comes to attaching great significance to 1-plus, 2-plus, or 3-plus "improvement" and attempts a determination of the "best" treatment on statistical analysis of such arbitrary data, one is building on a somewhat unstable foundation. Yet sweeping recommendations have often been made on no firmer basis than this. What is most unfortunate, however, is that a too great reliance on comparative regimen studies seems sometimes even to discourage independence of thought and to permit outmoded treatments to persist overlong.

For example, many of the papers on the antimicrobial therapy of tuberculosis which were published within the past year or two still recommended streptomycin and PAS as the "therapy of choice" in pulmonary tuberculosis. This recommendation was made even though it was recognized explicitly or implicitly in these reports that in the most *serious* forms, such as miliary and meningeal tuberculosis, it is *essential* to include isoniazid and that isoniazid is itself the most effective, the cheapest, and the least toxic antituberculosis drug available. Indeed, even while acknowledging these superior qualities of isoniazid, a great many experts insisted that it must *never* be used alone and that usually it should not be used in initial treatment at all. This is such doubtful reasoning that I might be accused at least of exaggeration, if not of misrepresentation. Yet it is difficult to see how one can draw any other inference from many, if not, indeed, from the majority, of the recent publications on this subject.

It is perhaps unjust to attribute all such inconsistencies to the influence of cooperative statistical studies, since the major ones which have been mentioned are admirably organized and the results are cautiously interpreted. Moreover, they *do* show, for the most part, that multiple-drug regimens, including isoniazid, are, as might be expected, generally more effective than streptomycin-PAS. This is true at least of the American studies in which streptomycin is usually given only twice weekly and PAS in a maximum dosage of 12 gm. daily. The point is not, however, that the controlled studies have merely confirmed what could be anticipated. It is rather that the influence of this type of investigation on clinical thought has become so great that clinicians generally have come to

distrust their own observations and judgment until these have received statistical sanction.

Little consideration has been given, until very recently, to even the *possibility* that different forms of tuberculosis, or different types of pulmonary tuberculosis, might be more satisfactorily treated by different drug regimens.[2] On present evidence, it seems highly probable that the best management of tuberculosis will prove to be, not a single drug regimen which is optimal for all cases (as seems to have been the goal and the orientation of most investigations up to the present time), but that *different* regimens will be found to best suit *different* forms and types of the disease. To determine such selective indications on a thoroughly scientific statistical basis, however, will require so much manipulation of groups and subgroups and so much time that it is not realistic to suspend action in this field until proof is at hand. The same may be said concerning the use of resectional surgery for various types of lesions, the use of pneumothorax or pneumoperitoneum, the duration and conditions of rest treatment, and decisions whether or not to insist on hospitalization. All, or most, of these questions should be systematically investigated, but it is unlikely that they all will be, or that unequivocal results will be obtained, in time to greatly affect patterns of action which will inevitably evolve in the meanwhile.

Accordingly, it appears to me that there is a great necessity at the present time to formulate a schema for the treatment of tuberculous disease. I believe that this can be done now on a rational basis, taking advantage of the established facts and, when necessary, attempting to anticipate the strongest probability of the future. Such a formulation has been conspicuously absent from current discussions and it is perhaps a hazardous undertaking. The present situation, however, borders so nearly on chaos and the emphasis has been so predominantly on the *disadvantages* of practically every type of treatment, that I undertake this self-imposed task without apology. It is presented for consideration and discussion, with no claim that it represents finality. It is indeed nothing more than a crystalization of my own thought and practice of the past year or two. If in some respects the schema appears to conflict with current majority concepts, it is not because of any light-hearted fondness for "heresy." Indeed, the belief that the present uncertainties and conflicts need to be resolved is accompanied by some sense of urgency. No attempt is made to go into details of dosage, which are well known, nor will there be more than passing mention of drugs which are still entirely in the investigative stage and are as yet unapproved for prescription sale in the United States.

MILIARY AND MENINGEAL TUBERCULOSIS

It is advantageous to head the schema with miliary and meningeal tuberculosis, not only because these are the most serious forms of the infection, but

[2] There are studies now in progress, both in the Veterans Administration and in the U. S. Public Health Service cooperative investigations, relating to the differential effects of various regimens according to the extent and character of pulmonary tuberculosis. The reports yet published are of a preliminary nature.

because there is little disagreement regarding the essentials of their treatment. There is no one, to my knowledge, who does not agree that isoniazid is *required* in the treatment of both miliary and meningeal tuberculosis. There is some question whether anything more than isoniazid is actually required in most cases of uncomplicated miliary disease. There is enough evidence to suggest, however, that isoniazid and streptomycin reinforce each other to some extent to make it prudent to use both of these drugs in uncomplicated miliary disease. The same situation obtains in meningitis with or without overt miliary dissemination. In meningitis, particularly, the maximal attainable antimicrobial effect is required because, despite the great reduction in mortality since the introduction of isoniazid, partial or total failures are not uncommon.

The intrathecal administration of streptomycin, however, does not seem to add sufficiently to justify its risks, at least as a routine treatment. In severe late cases, with cerebrospinal block, it may still have a place along with the intrathecal tuberculin treatment devised by the Oxford group. The intrathecal administration of isoniazid has not been thoroughly evaluated, to my knowledge, probably because to most it does not appear rational in view of the easy diffusibility of systemically administered isoniazid into the central nervous system and cerebrospinal fluid. More promising lines of investigation for improving results in meningitis appear to be in the concomitant administration of steroid hormones, or possibly of pyrazinamide-isoniazid. The use of the enzymes streptokinase-streptodornase and of trypsin, has been in the main disappointing. Judging from published reports, as I have had no personal experience with these latter methods, it is my belief that they may be dropped from practical consideration.

In summary, then, the preferred treatment of miliary and meningeal tuberculosis at the present time appears to be the administration of isoniazid and streptomycin. The question whether to add PAS as a third drug seems, somewhat paradoxically, perhaps, to be of less importance with respect to these inherently most dangerous forms of tuberculosis than in the so-called "open" forms of the disease. Bacterial drug resistance, as manifested by relapse under treatment, is a great rarity, if it occurs at all, in miliary and meningeal tuberculosis under isoniazid treatment. The addition of streptomycin is of importance for its additive effect with isoniazid rather than for its prolonging effect. The addition of PAS is, therefore, considered to be optional. The serious problem of cerebrospinal block in meningitis remains without a satisfactory solution. When block is demonstrated or probable, it is important to institute ancillary measures early. One possibly effective measure of this sort is the addition of steroid hormones, but it has not yet been conclusively shown that this is as effective as the Oxford treatment with intrathecal tuberculin.

PULMONARY TUBERCULOSIS

To formulate a schema of management of pulmonary tuberculosis is a larger task. Not only is there more diversity in the manifestations of disease than in the miliary-meningeal forms, but there is more uncertainty about the results and

interpretation even of the best controlled clinical studies. There is also more controversy. Nevertheless, there is little, if any, dissent from the paramount and inescapable conclusion that antimicrobial therapy has become the keystone in the management of pulmonary tuberculosis. It is agreed that all cases of active pulmonary disease require antimicrobial therapy continuously and for a long period; that is to say, for at least as long as one year. Here the agreement ends. There is sharp disagreement on the selection of antimicrobial agents; the necessity for hospitalization; the employment of collapse therapy; the indications for resectional surgery; the indications for, duration of, and even the definition of, adequate rest treatment and; finally, the significance of biologic changes in tubercle bacilli exposed to antimicrobial agents. It is not the purpose of the present paper to discuss in detail all of the evidence which bears on these important questions. Nevertheless, certain observations should be mentioned because they appear to bear directly on the treatment choices which are suggested.

Isoniazid alone has been almost universally condemned for the treatment of pulmonary tuberculosis. As is well known, it has not even been extensively investigated for long-term treatment. Such evidence as is available, however, indicates that it is approximately as effective as streptomycin-PAS. Bacterial resistance, as determined *in vitro*, does proceed approximately as with streptomycin alone. But the clinical correlations are less uniform. Combinations of isoniazid with streptomycin or with PAS appear to delay the emergence of resistance, similarly to the combination of streptomycin with PAS. These combinations also appear to improve the clinical results to a certain extent, at least in the early months of treatment, suggesting an actual enhancement of the total antimicrobial effect. When maximal antimicrobial effect is clearly desirable, as in extensive caseous pneumonia, multiple-drug therapy, including isoniazid, is most probably to be preferred to isoniazid alone. In less threatening forms of pulmonary tuberculosis, however, the preference for a multiple-drug regimen is more debatable. Not only is there a possibly unnecessary increase in the risk of toxic side effects, but a possibly valuable conservation of therapeutic resources is disregarded by adherence to a strict policy of multiple-drug therapy. Such multiple-drug therapy only delays, it does not prevent, bacterial resistance. There is accumulating evidence, furthermore, that, within reasonable limits, the postponement of resectional surgery until late in the course of long-term drug therapy diminishes the hazards of the surgery, and that fewer and less extensive procedures may be needed. By the same token it is important to have dependably effective antimicrobial therapy available at the time of surgery. Moreover, as no regimen is known to be truly eradicative of tubercle bacilli, future relapses of the disease cannot be discounted.

These considerations have led me to prefer the use of isoniazid alone, not only in most cases of minimal pulmonary involvement, but in some cases of moderately or even far advanced disease.

The selection of advanced cases for isoniazid as single-drug therapy is admittedly difficult. The ideal cases are of two varieties. The first variety is that

of the patient with an early and not very large cavity and with not too extensive disease. Such a patient may do very well on isoniazid as single-drug therapy, and thus one conserves streptomycin-PAS, as a unit, to use with surgery later should surgery be necessary. The second variety, in which isoniazid alone seems rational for initial therapy, consists of patients with old but fairly localized cavitary disease of mixed type, with some irreversible components which will most probably have to be resected, and which is, *because* of the irreversible components, unsuitable for collapse therapy. By using isoniazid alone for initial therapy one may perhaps not arrive at the optimum time for resection so early as with multiple drug therapy, but there is less uncertainty about selecting this time as there is little danger of missing it because of the possible emergence of multiple-drug resistance.

These two categories of advanced disease in which isoniazid alone as initial therapy seems advantageous comprise together but a small minority of cases, but it seems to me an important minority. In a somewhat limited experience, to be sure, our group has observed that even in *unselected* advanced cases initial therapy with isoniazid alone is not unsatisfactory and that the incidence of deterioration under such therapy is little, if at all, higher than under other regimens. This was also brought out by the U.S. Public Health study and more recently, I believe, by Dr. Tucker's re-analysis of earlier data of the Veterans Administration study.

As to the results of isoniazid alone in minimal pulmonary tuberculosis, I know of no reported study limited to this group. My personal experience of the last two or three years, during which I have used isoniazid alone almost exclusively in minimal cases, has given me no cause to regret this choice. In advanced disease, apart from the two special categories which I have already referred to, I prefer a two-drug regimen.

I believe that the preponderance of evidence indicates that there is some advantage in using streptomycin or PAS along with isoniazid, even though this advantage appears to be small and is not yet absolutely established. There is little indication at this time which is the preferable drug, streptomycin or PAS, to use as a companion to isoniazid. There are some theoretical objections, at least, to the isoniazid-streptomycin combination, because it commits the two most effective agents simultaneously. I have not been greatly impressed by these objections for the following reasons. In practice the combination is very effective, and approaches, if indeed it does not equal, the combination of isoniazid and pyrazinamide, which, according to experimental evidence, is probably the most effective multiple-drug therapy. The "sputum conversion" rate with isoniazid-streptomycin in well-studied series is high. In my own experience, "conversion" occurs not infrequently even with persisting cavities, and delayed surgery is attended by fewer complications than might be anticipated on theoretical grounds. Finally, there are maintained in reserve both viomycin and PAS which may be effectively used together, whereas, if streptomycin is the drug held in reserve, there is no truly satisfactory companion drug for it if PAS resistance has become a factor.

The advantages of isoniazid-streptomycin over isoniazid-PAS are not, how-

ever, so considerable that the latter may not be the preferred selection, particularly when there are special reasons for avoiding streptomycin. The most important of these reasons, aside from hypersensitivity to streptomycin (which cannot be anticipated), pertains to situations in which it is most important to avoid eighth-nerve toxicity even of small degree. This is particularly important in the elderly or in patients with pre-existing auditory impairment. In most other situations, PAS toxicity is more often a potential problem than streptomycin toxicity. The use of all three drugs together appears, on present evidence, to have no advantage in the treatment of pulmonary tuberculosis. On the contrary, it has a demonstrated disadvantage because it is the least well tolerated of the multiple-drug regimens.

Pyrazinamide-isoniazid, although it has shown marked effectiveness, is still not a regimen to be recommended because of the danger of hepatic toxicity from the pyrazinamide, at least in the dosage of 50 milligrams per kilogram. Whether in lowered dosage of pyrazinamide this combination will be safe and equally effective is yet undetermined, but I find little reason for optimism in this respect.

In summary, therefore, the best choices of initial antimicrobial therapy in pulmonary tuberculosis appear to comprise *isoniazid alone*, *isoniazid-streptomycin*, and *isoniazid-PAS*. Isoniazid alone is, I believe, the preferred choice in minimal and in certain selected cases of advanced disease. For the remainder, the choice between isoniazid-streptomycin and isoniazid-PAS can be determined largely on practicability and consideration of potential toxicity in particular situations. In the youthful, I prefer streptomycin and, in the severely ill, I prefer to give it daily rather than twice weekly. In the elderly, I prefer to avoid it, save for the most compelling indications for maximal antimicrobial effect.

* * *

The treatment of pulmonary tuberculosis does not, of course, begin and end with the administration of antimicrobial drugs. That they are more effective than bed rest alone in arresting the process of tuberculosis, I believe no one will deny. It does not necessarily follow from this, however, that bed rest can be altogether discarded. We have indeed scarcely any information on the extent to which bed rest can be omitted or modified in the early phases of treatment. It is conceivable that it may prove to be of little or no advantage, although there are compelling reasons to think that until the time of cavity closure and "sputum conversion" it should be maintained, at least in modified form. This does not imply, however, that recalcitrant patients who refuse hospitalization may not benefit from active drug therapy or that such therapy should be withheld either from these or from patients awaiting hospitalization. With several effective drugs now available, and the possibility of substituting different combinations for others, there is indeed little justification for withholding treatment from patients who for one reason or another cannot be immediately hospitalized.

* * *

The indications for surgery in pulmonary tuberculosis are as much a matter of disagreement as the selection of drug regimen, if not even more so. There is, however, little disagreement on certain indications. Persistence of cavities and

of "positive sputum" for six months or longer is quite generally regarded as an indication for resection, if the lesions are thought to be resectable. Persistent small cavities with repeated "negative" cultures, however, are less uniformly resected than they were formerly, particularly in instances in which it is difficult to differentiate between true cavities and emphysematous bullae. When roentgenographic rarefactions are fairly obvious residuals of originally typical cavities, or when the tomograms show other unequivocal evidence of cavity, I still prefer that they be resected or receive other treatment appropriate for a persistent cavity.

The principal controversy of the past several years has been about the resection of residual closed lesions, whether they be filled-in cavities or other necrotic residues. The enthusiasm for resection of these lesions appears to be waning, and I believe that this trend is in the right direction. The larger of such lesions may best be resected, but even in these there is much to be said for waiting until there is some evidence that they are giving trouble.

The entire character of tuberculosis during and often for long periods after antimicrobial treatment has changed from the way in which the disease formerly behaved. Acute and disastrous exacerbations which cannot be rather promptly controlled have become the exception. The disastrous sudden "spreads" which we used so greatly to fear exist more in our memories than in our present experience. They may occur in patients not well followed or well regulated in their post-hospital course, but such patients are in danger, whether or not their more conspicuous residual lesions have been removed. This, at least, is my belief from my own experience of the last several years. I think the burden of proof to show the necessity for this type of prophylactic surgery is on those who follow the procedure. To point to low mortality and low incidence of serious complications is not, in my opinion, sufficient to justify preventive major surgery in a period, such as the present, when the mortality from the *disease* is falling so precipitously. In a paper published last November, Dr. Johnsen and Dr. Peck reported that in 1953 there occurred in the North Carolina Sanatorium only 29 deaths as compared with 154 in 1948; and only about half of these 29 deaths were actually a direct result of progressive tuberculosis. The decline in the general tuberculosis mortality indicates that the experience noted at McCain is not an isolated one, but that the same trend is to be found everywhere. Tuberculosis has become a much less frequently fatal malady than it was formerly, a fact which should not be neglected in calculating the risks of therapeutic measures.

Similarly, the reversibility of tuberculous disease in the lungs is far greater than was formerly appreciated. Conservation of possibly useful lung is, therefore, never to be disregarded. There has, indeed, been a widespread realization of this principle which is implicit in the trend to resectional surgery in preference to nonreversible collapse procedures. Thoracoplasty is, in my opinion, to be avoided whenever possible except as needed in modified form in conjunction with resectional procedures. We have performed scarcely any conventional thoracoplasties in the past four or five years. When an irreversible collapse procedure seems the only surgical solution, the modified plombage type of

thoracoplasty has been our preferred procedure. Thus far, at least, we are not disillusioned with it.

The principle of conserving functional lung tissue is not observed to its full extent, however, merely by substituting resectional procedures or modified thoracoplasties for the old conventional thoracoplasty. It is necessary to limit *all* surgery as much as possible. I recently saw a patient who had become functionally severely handicapped by extensive secondary surgery resulting from the complications of multiple resections of closed or so-called residual lesions. This is a patient who prior to surgical intervention was clinically well. Such results, even if they be few, are as deplorable as operative deaths from procedures which are of debatable preventive value.

Apart from the question of resection of residual localized lesions, there is a problem relative to resection of more extensive residual disease and so-called "destroyed" segments. Often on pathologic examination the destruction is less than was anticipated, and evidence even of any specific tuberculous disease may be surprisingly little. In the absence, therefore, of continued clinical manifestations of active tuberculous disease in the form of persistent output of bacilli, the case for radical surgery is becoming weaker on all counts. Even in the presence of definite cavitation and persistent bacillary output, a trial of reversible collapse procedures merits more attention than it has received in recent years. There is abundant evidence, principally from abroad, that the formerly so greatly to be feared complications of pneumothorax are markedly reduced by concomitant antimicrobial therapy. Rather than to be completely discarded, as it is in many clinics, this procedure deserves at least a limited renascence. Except for unusually large or obviously fibrous-walled cavities and except in the presence of stenotic bronchial involvement, the indications might well be those currently accepted for resection of cavitary disease. Only the most satisfactory pneumothoraces would need to be continued, and even so much major surgery might thereby be avoided.

EXTRAPULMONARY TUBERCULOSIS

The same principles which govern the treatment of pulmonary tuberculosis may be applied to most forms of extrapulmonary tuberculosis. I do not propose to discuss them all, or any in great detail. Laryngeal and intestinal tuberculosis, which exist principally as complications of pulmonary tuberculosis, are most often promptly healed by the antimicrobial therapy administered for the pulmonary disease. Any special problems rarely arise. We now know, mainly through the meticulous work of one man, Dr. John Lattimer, that genitourinary tuberculosis is no longer to be approached as an immediate surgical problem. Multiple-drug antimicrobial therapy is very successful. In contrast to pulmonary tuberculosis, however, the latest evidence suggests that the use of all three drugs, rather than any combination of two, gives the best results. Isoniazid, streptomycin, and PAS, together, have in Dr. Lattimer's experience produced sterilization of the urine which has, so far, lasted twelve months after termination of a year's therapy in twenty cases of advanced bilateral renal tuberculosis. This raises the question whether nephrectomy is indeed ever required in the

treatment of renal tuberculosis, although longer follow-up is still required to definitively answer the question. The experience indicates very definitely, however, that *early* surgery should not be undertaken, a fact which seems not yet to be appreciated by many urologists. It also suggests that in this form of tuberculosis the preferred regimen is probably the triple-drug regimen.

In contrast to the situation with renal tuberculosis, the recent literature on skin tuberculosis, even in its formerly most intractable form of *lupus vulgaris*, is usually very well taken care of by isoniazid alone.

The evidence is not so conclusive with respect to tuberculosis lymphadenitis, but our experience suggests that isoniazid alone is usually adequate in this form of tuberculosis as well. Dr. Katharine Butler of our clinic has followed twenty-five cases of tuberculosis of the superficial lymph nodes treated with isoniazid alone, only two of which relapsed under treatment. Both of these patients had exacerbations at about one year of treatment, after they had become fully ambulatory. Whether better results would be obtained by multiple-drug therapy we do not know, nor have I seen any reports which indicate this. The place of radiation or excision in conjunction with the chemotherapy of tuberculous lymph nodes is not determined. There is, however, little to suggest that *early* surgical excision of lymph nodes is necessary or often desirable.

The status of bone and joint tuberculosis is not well defined, either with respect to the optimum drug regimen or the extent to which surgery is still indicated. There is much evidence, however, that considerable healing may take place under antimicrobial therapy, that associated cold abscesses may resorb to a considerable extent, and that they may be incised or excised when necessary with much better results than formerly.

The status of genital tuberculosis in the female is likewise poorly defined. I should like to see more conservatism than is yet practiced, as I doubt that all of the female internal genitalia need to be sacrificed as frequently as they still are by most gynecologic surgeons when they find pelvic tuberculosis.

Tuberculous peritonitis usually responds well to isoniazid or any regimen containing isoniazid. In the early case, particularly, isoniazid alone seems adequate. This I believe is also adequate in tuberculous pleurisy with effusion.

Finally, there is the question of primary tuberculosis, whether overt or manifested only by the recent development of a skin reaction to tuberculin. Without going into detail, since I think the rationale is obvious, I favor treatment of all primary tuberculosis in infants, and I think that isoniazid alone is probably adequate. In older children, in adolescents, and in adults, the case for treating the recent "convertor," or tuberculin reactor, is less compelling because, among these groups, miliary and meningeal disease are less common early sequels of the first tuberculous infection than they are in infancy. If reactors in the older groups are to be treated, I see little reason to commit other drugs than just isoniazid, or indeed to subject such persons to the rigors of multiple-drug therapy.

* * *

In closing, I wish only to repeat what I suggested earlier, that a careful consideration of presently available evidence suggests the desirability of formu-

lating a schema of treatment which is selective and, as nearly as possible, optimal for each clinical variety of this disease of such diverse manifestations. The rationale for each of the proposals I have made in attempting such a formulation is based only on an individual, and therefore personal, evaluation and interpretation of evidence garnered from many sources. Many will disagree, I am sure, with much that I have suggested. Some, doubtless, will feel that the attempt is altogether premature and that the entire field of tuberculosis therapy is still too much in flux to permit any encompassing plan. Nevertheless, the attempt may be of some value if only to focus attention on the numerous unsettled questions and to make our *practice* more systematic and less arbitrary, while still continuing the methodical *investigation* of tuberculosis therapy.

THE ACOUSTIC BASIS OF THE CHEST EXAMINATION[1]

Studies by Means of Sound Spectrography

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INTRODUCTION

More than a decade ago Potter and his associates (1) at the Bell Telephone Laboratories devised a method for simultaneously recording, analyzing, and displaying three variables of speech sound: time, intensity, and frequency spectrum. The method, in this original application denominated "visible speech," permits highly refined and graphic analyses of vibrations in the audible range. The use of the Bell sound spectrograph in the study of cardiovascular sound ("spectral phonocardiography") has been illuminating (2–6). Spectral phonocardiograms look the way the heart sounds; they match closely the mental image of heart sounds and murmurs. They have been useful in teaching clinical auscultation of the heart. As spectral phonocardiograms are detailed and three-dimensional analyses of sounds, they display quality (timbre, Klangfabre), are ideal for following the course of some varieties of heart disease and the effects of cardiovascular surgery, provide an excellent medium for presentation of these sounds on the printed page, and represent a valuable tool for the investigation of many clinical and physiologic problems concerning cardiovascular sound.

Acoustic phenomena—the percussion note, breath sounds, rales, voice sounds—constitute the predominant part of the physical examination of the lungs. The investigations described below were undertaken to determine the physical nature of the variations in thoracic acoustics on which diagnostic conclusions are founded. A detailed analysis of the changes in frequency and intensity characteristics of respiratory sounds permits deductions concerning the physical alterations which have been produced by disease. It was hoped that such studies would increase the diagnostic usefulness of these acoustic signs and provide valuable devices for the teaching of physical diagnosis.

METHODS

In figure 1 the principle of a sound spectrograph which provides direct, i.e., instantaneous, analyses is illustrated. Sound from the chest of the patient is picked up by means of a condenser-type microphone (Altec 21BR 150). This sound, in electrical form, is passed through a battery of filters, each of which is tuned to a specific pass-band. The output from each filter, representing the energy in a specific frequency band, activates the marking unit of the recorder, which in figure 1 is represented as a tiny light. It is the play of the battery of lights on the recording film which produces the sound spectrogram. Note that *time is on the abscissa* and *frequency on the ordinate*. *Intensity* (loudness) *is in-*

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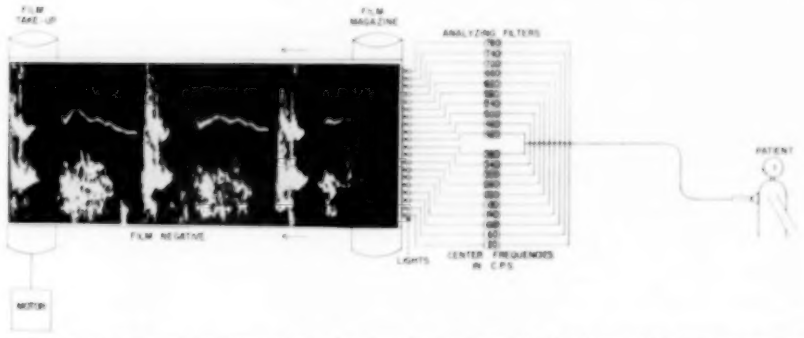

FIG. 1. Schematic representation of a sound spectrograph which provides instantaneous displays. The spectrogram in this illustration is that of the sounds in a case of bronchial asthma.

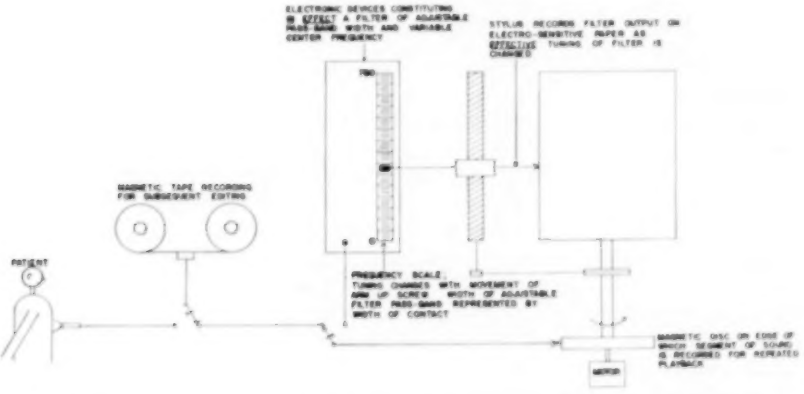

FIG. 2. Schematic representation of the sound spectrograph used in these studies

dicated in this case *by degree of brightness* of any given position of the record, inasmuch as the brightness of each bulb is a function of the energy content in each frequency band.

> The specific example of sound in the diagram in figure 1 is a recording from an asthmatic patient. Although not labeled, the greatly prolonged expiratory phase is easily identified. The musical quality of the breath sounds is displayed by the harmonics present. A more detailed exposition is presented below.

In constructing an instrument of the type shown in figure 1, it is obviously expensive and difficult to provide the large number of individual filters necessary for a refined analysis. Therefore, the sound spectrograph used in the present studies was developed along the lines indicated in figure 2. Recordings are first made on magnetic tape. These are then edited and desirable sections are picked

out for analysis. Such a section is played onto the magnetizable rim of a disc, which is mounted on the same axis as the kymograph drum. This segment of sound is then played back repeatedly through an electronic device which *in effect* is a single filter, the tuning of which is changed progressively with the repeated playbacks. Recordings are made on electrosensitive paper (or more recently on photosensitive paper). A mechanical link provides for progressive change in the *effective* tuning of the filter set-up as the writing arm moves up the screw. It should be appreciated that what is in essence a single filter has not only a variable center pass-band frequency but also an adjustable pass-band width.

When it seemed desirable for the sake of graphic display to have a simultaneous demonstration of respiratory phase, respirations were registered on the magnetic tape and on the finished spectrogram by means of a frequency-modulated carrier.

OBSERVATIONS

Percussion Notes

The characteristics of tympanitic, resonant, and dull percussion notes are compared in figure 3. The three have in common a central core which is the sound of impact of the percussing finger on the plessimeter finger. This impact sound constitutes a considerable part of what one hears in the percussion note. In the case of the completely flat note, there is little else because the blow is not able to set the structures under the percussing finger into vibration to any significant extent. In the case of the resonant note, vibrations, maximal in the range of the natural frequency of the thorax, are superimposed on and follow the impact sound. The natural frequency of this subject's thorax (a normal young male adult) is seen to be approximately 140 cycles per second (c.p.s.).

Comparing the resonant and the dull notes, it appears that the former is louder and longer than the dull note as a result of the additional forced vibrations of the normal air-filled thorax. The dull note is, on the average (and in the way it impresses the ear), of a higher pitch. This is not principally because vibrations of higher frequency have been excited, but because the average pitch of the dull note is not weighted by the low-frequency vibrations of the thorax. It is true that, in the relatively dull note of pulmonary consolidation, vibrations of higher frequency and lesser intensity are incited in the pathologic tissue which has a higher natural frequency.

Comparison of the tympanitic note, e.g., over the stomach bubble, with the other notes reveals that the main difference is a more musical quality which is reflected in these displays by the presence of more clearly defined harmonics. There is a distinct fundamental at 180 c.p.s., and the second harmonic at 360 c.p.s. is equally well defined.

Early Studies of Percussion Notes

The lesser *intensity* of the dull percussion note as compared with the resonant was demonstrated by Selling (7), in Friedrich Muller's laboratory, by a simple but ingenious method

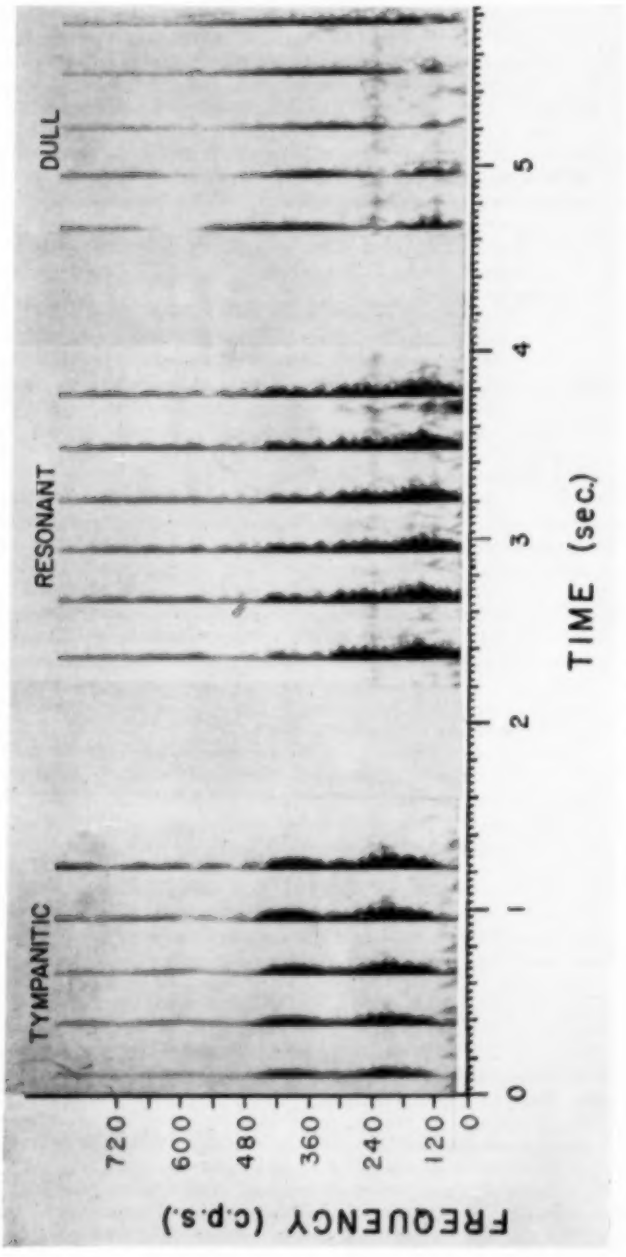

FIG. 3. Percussion notes

He compared the maximum distance at which each type of note could be heard when produced by a physician percussing over the lung and over the thigh with equal force. He found that the resonant note is audible approximately five times as far as the dull note. Since intensity is inversely proportional to the square of the distance, it can be concluded that the healthy lung encased in the thoracic cage is approximately twenty-five times more resonant than the muscle mass of the thigh. Selling and Scripture also recorded percussion notes on phonograph cylinders of the Edison type and demonstrated by microscopy that the cut produced by the resonant note was considerably deeper than that produced by the dull note. In an even more definitive experiment, Selling and Edelman measured the intensity of these notes by means of the Einthoven string galvanometer and showed that the dull percussion note was of considerably lesser intensity. The greater *duration* of the resonant note was likewise demonstrated by Selling's studies by both the phonographic and the Einthoven galvanometric method.

The timbre of the resonant or vesicular percussion note was compared by Thatcher (8) with the timbre of the note produced by striking a loaf of bread covered by its crust—the air cells of the bread are analogous to the alveoli. Rist (9) observed that, when milk is stirred as it is brought to a boil, the stirring produces a resonant note when the boiling point is reached. Again, the boiling milk contains innumerable tiny bubbles filled with vapor under pressure and separated by liquid partitions endowed with a certain amount of elasticity. Geigel (8) compared the tympanitic percussion note with that produced by percussing a glass cylinder filled with very frothy beer.

Placing the pitch of percussion notes on the musical scale is difficult because they are more nearly noises than musical notes. Nevertheless, an average pitch can be determined, particularly by comparison with the pitch of other notes, just as each of the kettledrums of an orchestra emits a noise of distinct average pitch and a melody can be played on a series of glass tumblers. Augenburgger and Skoda noted the variations in pitch of different resonant percussion notes, and Austin Flint (10) emphasized this fundamental acoustic characteristic. Gerhardt in 1876 (11) and Selling in 1907 (7) used Helmholtz resonators for studying percussion sounds. Selling found it necessary to use cylindro-conical resonators as much as 2.0 meters in length to study the most low-pitched of the percussion notes. These studies and those of Martini (17) and of Rist (9) demonstrated a series of tones ranging from a pitch corresponding to F below low C (approximately 80 c.p.s.) in the musical scale up to C above high C (approximately 800 c.p.s.), and depending on the volume of the thorax (*see* below). Martini (17) placed the fundamental of the natural frequency of the child's lung at 170 c.p.s.; of consolidated lung, at 140 to 190 c.p.s.; of normal adult lung, at 100 to 120 c.p.s.; and of emphysematous lung, at 70 to 90 c.p.s.

The character of the plessimeter impact sound is determined by the nature of the plessimeter. The ivory and wooden plessimeters of an earlier period were shrill and noisy because they were stiff. In digito-digital percussion, now used almost exclusively, the impact note is less striking, yet clearly discernible. It is this plessimeter impact note which is heard predominantly in the dull percussion note and exclusively in the absolutely flat percussion note. According to Selling, a plessimeter of soft rubber is almost silent.

Another older method by which the frequency composition of percussion notes was studied many years ago by Cartex (12) is that which involved photographing manometer flames. In this method, devised by Koenig (*see* Miller (13)) in 1862, the gas pressure to the flame is modulated by a delicate membrane which vibrates with the sounds to be studied. Variations in the height of the flame are what are photographed on moving film to produce a record of the sound vibrations. Selling applied Scripture's method to this problem (*see* Miller (13)). Oscillographic-like tracings were made by a machine which took the vibrations of the gramophone disc and, after passage through a system of delicate compound levers, marked them on the smoked paper of a kymograph.

Montgomery (14) states that the "fundamental," if such it can be called, of vesicular (resonant) percussion and of normal vesicular breathing is approximately 108 c.p.s., a close match with 128 c.p.s., the fundamental of man's voice. The fundamental of a woman's voice is closer to 256 c.p.s.

It is a general experience that tympanitic percussion notes are more musical than the resonant ones; their pitch is more easily placed in a musical scale. Selling's string galvanometer recordings of tympanitic notes (7) displayed more regular vibrations than were found in resonant notes which produced complicated oscillograms. The same finding was made by May and Lindemann (reported by Fahr (15)), who employed records in an arduous way. They obtained an artificial membrane by plunging a metal ring into a soapy solution. This delicate membrane vibrated in a highly sensitive manner with sound vibrations and, when so placed that it reflected an image on moving photographic film, it inscribed an oscillogram of the sound.

The Natural Frequency of the Thorax

It is clear from the previous exposition that the thorax has a natural frequency[2] which is demonstrated by an analysis of percussion notes. This natural frequency in general varies inversely with the volume of the thorax. When a subject sings a low note, the hand placed on the chest will feel the vocal vibration; the thorax behaves like a Helmholtz resonator. If the subject sings an ascending scale, the vocal thrill, palpated on the surface of the chest, will become weaker and disappear. The threshold is higher when the singer is in expiration. The matching of the pitch of the voice to the volume of the thorax is a determining factor whether vocal resonance is present or not. Vocal resonance is the rule in men because of good match. In women vocal resonance is rare because, although the natural frequency of the thorax is higher, the voice is even more shrill than the thorax can match. On the other hand, infants and children are likely to show conspicuous vocal resonance because of having close to ideal match of their more shrill voices to the higher natural frequency of the thorax.

In the strict sense, the thorax is a double coupled system comprising, on one hand, the thoracic wall, particularly the rib cage, and, on the other, the lungs. The natural frequency of the stiffer chest wall is somewhat higher than that of the enclosed structures.

The natural frequency of the thorax is an important factor in the manner in which murmurs are altered in quality in transmission to various parts of the chest. It has been noted that those frequency components of a heart murmur which are in the same general range as the natural frequency of the thorax tend to be transmitted best to various areas of the chest (3). An experiment comparable with this one of nature is to place a series of tuning forks one after another on the anterior portion of the chest and, by palpation, gauge the extent to which these vibrations of pure pitch are transmitted to the back. (In actuality, satisfactory results from this experiment (24) are vitiated by circumferential conduction of the vibrations in the rib cage.) Disease processes in the lung and pleura may have the result that the thorax, or a part thereof, resonates differently from normal. The lower-pitched components are diminished in intensity, and one says that the percussion note is higher pitched. This conclusion is due in part to the fact that the more shrill plessimeter impact note is left to impress

[2] Natural, specific or intrinsic, vibrational frequency depends on the tendency of an elastic structure, when deformed from its position of rest or equilibrium, to return to that position. The character of the structure's vibration during the period it is resuming its position of rest is determined by its modulus of elasticity: frequency is directly proportional to density and inversely proportional to volume.

the auditory perception to a greater extent. To some extent, also, the higher pitched note is the result of a higher natural frequency of resonation of the diseased thorax. Alterations in this frequency of resonation are important factors in the genesis of "E to A change," changes in breath sounds, and other phenomena discussed below.

Breath Sounds

The character of the breath sounds, as detected by stethoscope or microphone at the surface of the thorax, is determined to a considerable extent by the resonating and sound-conducting properties of the lung tissue between the larger airways and the point of detection. The average pitch of normal vesicular breath sounds varies in the same manner as the percussion notes, being progressively lower in infants, adult females, adult males, and patients with emphysema. The intensity of breath sounds is progressively decreased in the same series because of the greater distance between generator and pickup with the greater thoracic volume.

Normal vesicular breath sounds are demonstrated in figure 4. Expiration is relatively noiseless. Electrical interference at 60 c.p.s. is present.

The inspiratory "murmur," or normal vesicular breath sound, has its origin at the alveoli (16) and is the result of turbulence created when air currents spread out into the myriads of air sacs. It is well heard at the surface of the chest because it is generated at close hand. Movement of air in the larger airways is not necessary for its production. One variety of cardiopulmonary murmur is of fundamentally the same origin and has the same character as the vesicular sound (5). Other evidence for the vesicular origin of the vesicular inspiration sound (note the appropriateness of the terminology) is provided by the auscultation of exenterated lungs (16) and of hernia of the lung (18). The expiratory sound, on the other hand, has its origin at bifurcations in the larger airways by a mechanism comparable to the labial pipe of the church organ (17). Because of the porous, sound-absorbing intervening lung tissue, it is not surprising that the expiratory sound should normally be poorly heard at the surface of the chest.

In the breath sounds recorded from the immediate vicinity of the trachea (figure 5), the properties of the thorax are not the major controlling factors. The pitch of the sounds, which are actually noises, in inspiration and expiration is roughly the same, but the expiratory sound is appreciably louder. The writers have observed variation in the frequency level of the "center" of the tracheal breath sounds. These variations appear to depend upon the dimensions of the trachea, both length and caliber. As might be expected, the average pitch of the tracheal sounds is lower in persons who appear to have larger tracheas, and vice versa.

When pneumonia produces changes in the sound-conducting properties of lung tissue, expiration, which is ordinarily relatively noiseless, becomes audible and characteristic bronchovesicular respiration (figure 6) develops. Again electrical interference at 60 c.p.s. is present, as well as some at 120 and 180 c.p.s. It may be noted that, with partial consolidation, it is still only the lower-pitched

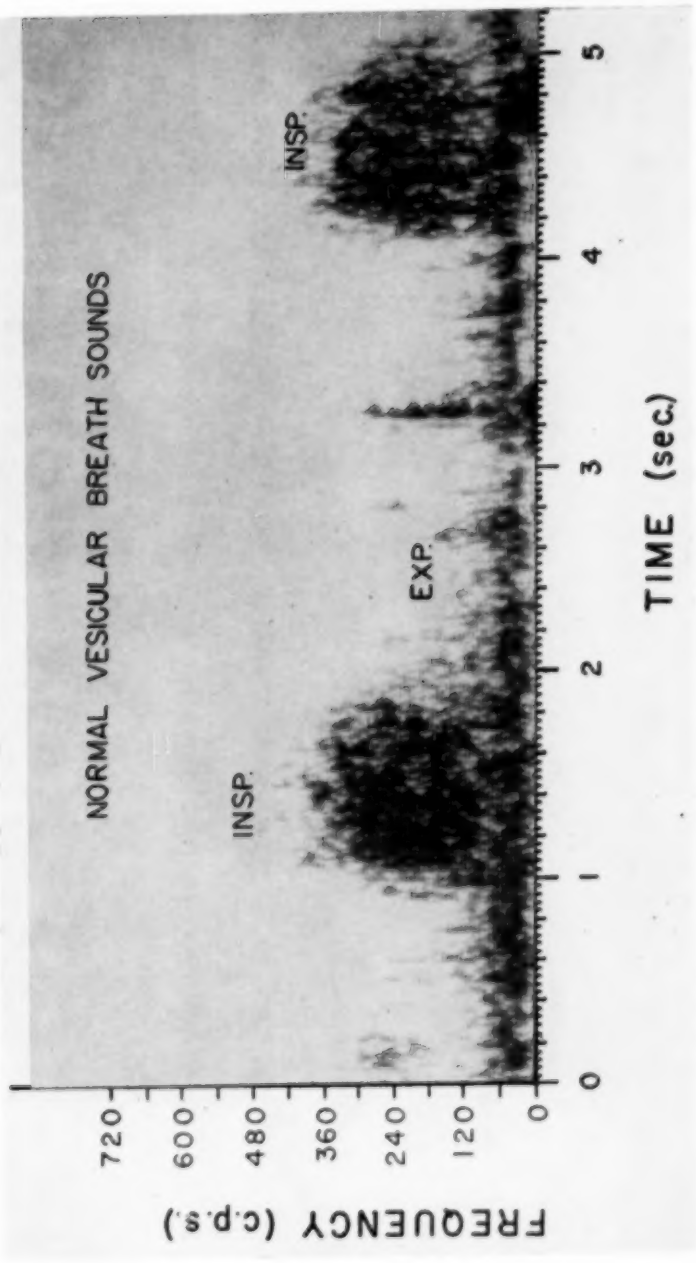

FIG. 4 Normal vesicular breath sounds

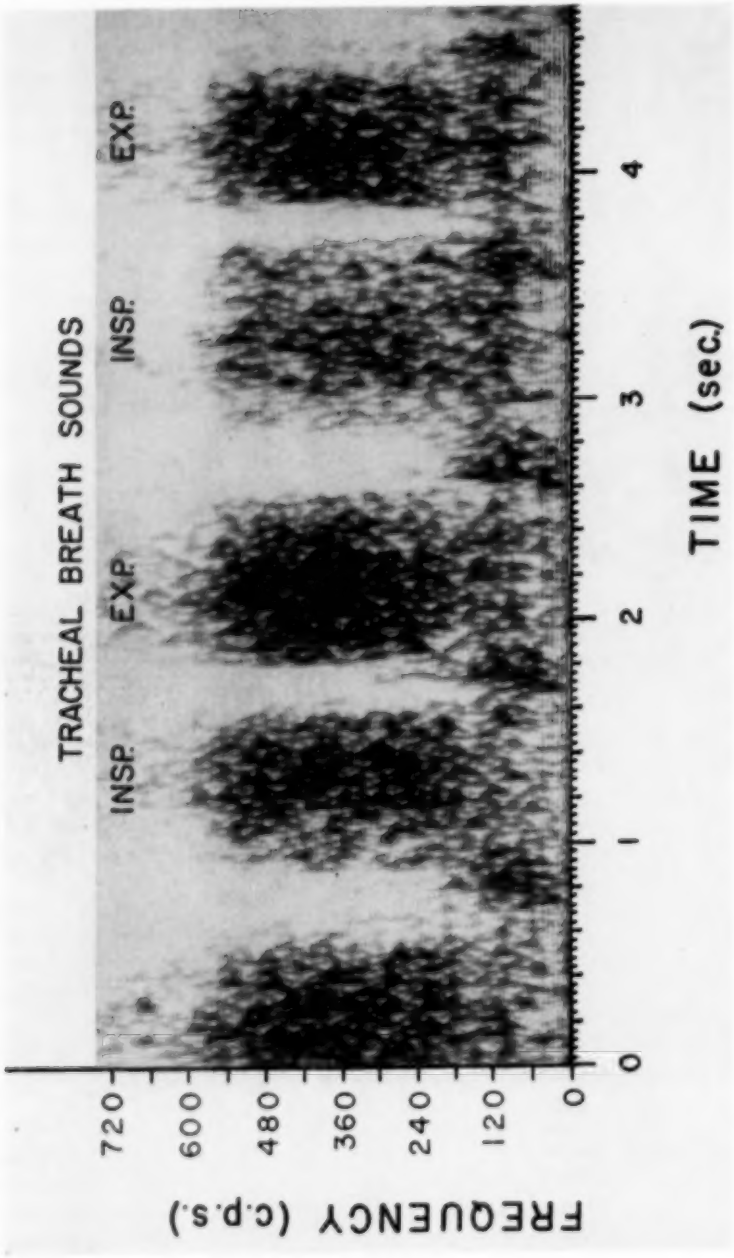

FIG. 5 Tracheal breath sounds

portion of the tracheobronchial sounds which are heard at the surface of the thorax. At this stage, also, the vesicular inspiratory sound is likely to be diminished, which is presumably the case in figure 6, since many of the alveoli are not functioning because of the exudate.

Further consolidation of pulmonary tissue, such as occurs in later stages of pneumococcal pneumonia, results in the audibility at the surface of the chest of the vibrations produced in the tracheobronchial tree. In experiments which are now classical, Martini and Mueller (19) determined the natural vibrational characteristics of the tracheobronchial tree at its several levels (*see* graphic representation in reference 15). These studies indicate that sounds produced by the mechanism of the labial pipe at the levels of the tracheobronchial tree where the diameter of the tubes is 3 to 10 mm. in diameter, i.e., any point below the bifurcation of the trachea, will have a frequency in excess of 1,000 cycles per second. Consolidated lung tissue, because of its better conducting properties, permits the detection of these vibrations at the surface of the chest. The result is so-called bronchial, or tubular, breath sounds. These are demonstrated in figure 7. In the example demonstrated, an additional feature of interest is the presence of heart sounds, confined largely to the frequency range below 240 c.p.s. Because the pneumonia in this case was in the left lower lobe, the heart sounds were transmitted to the microphone with abnormal clarity. Pronounced tachycardia was present.

Martini and Mueller (19) demonstrated in models that pronounced damping of sound vibrations occurs in tubes of 2 to 3 mm. in diameter. In tubes of 1 mm. in diameter very little conduction occurs. This was the basis for their conclusion that the bronchial breath sounds have their origin in bronchioles larger than 4 mm. in diameter. At this level the bronchiole has a cartilaginous wall, and its natural frequency would be expected to be little influenced by the presence of surrounding alveolar consolidation. The appearance of bronchial breathing with the development of consolidation is a matter of transmission of vibrations which occur normally. Anatomic studies revealed that, in the adult, thorax bronchioles of 2 to 3 mm. diameter lie 3 to 4 cm. from the surface of the chest and, in some areas, 5 cm. Near the vertebral column in the interscapular areas, these smaller airways may be as close as 1 to 2 cm. to the surface. These measurements are an indication of the depth of consolidation which is necessary for the development of bronchial breathing.

Using Helmholtz resonators, Martini and Mueller (19) demonstrated components of approximately 1,000 c.p.s. in bronchial breath sounds. In the experience of Cabot and Dodge (20), the highest frequencies lay in the range of 660 to 1,000 cycles per second.

Changes in Whispered Sounds

Corresponding to the presence of bronchovesicular breath sounds, the whispered voice sounds may be exaggerated in intensity and frequency span, as is illustrated in figure 8.

When bronchial breath sounds are present, the whispered sounds (21) take on

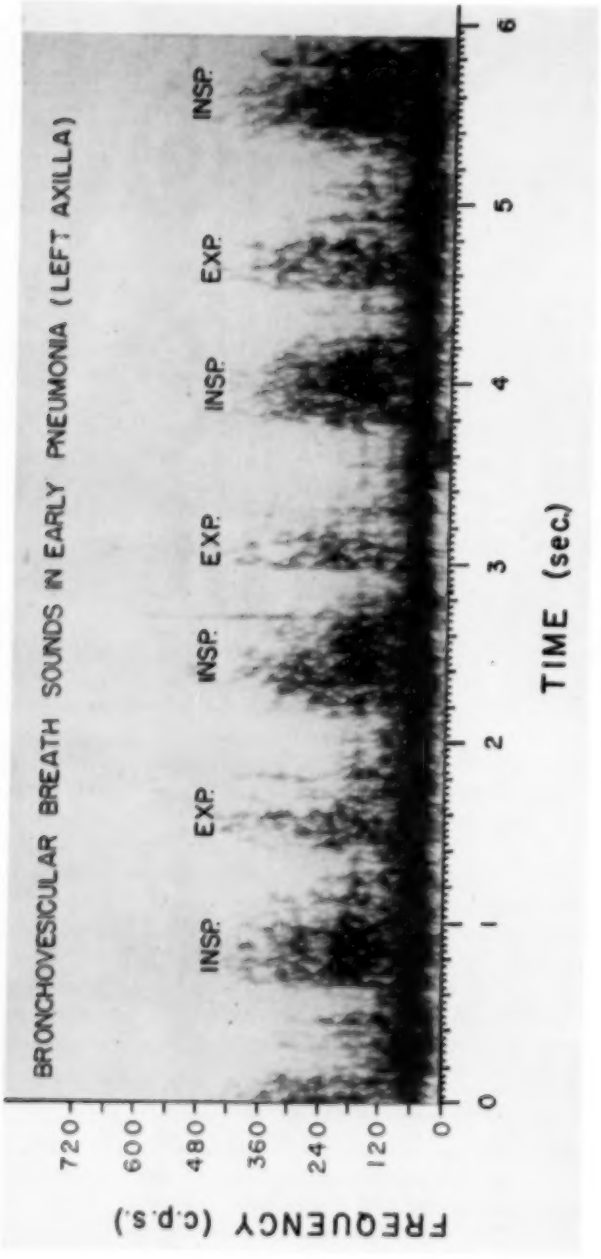

FIG. 6. Bronchovesicular breath sounds in early pneumococcal pneumonia

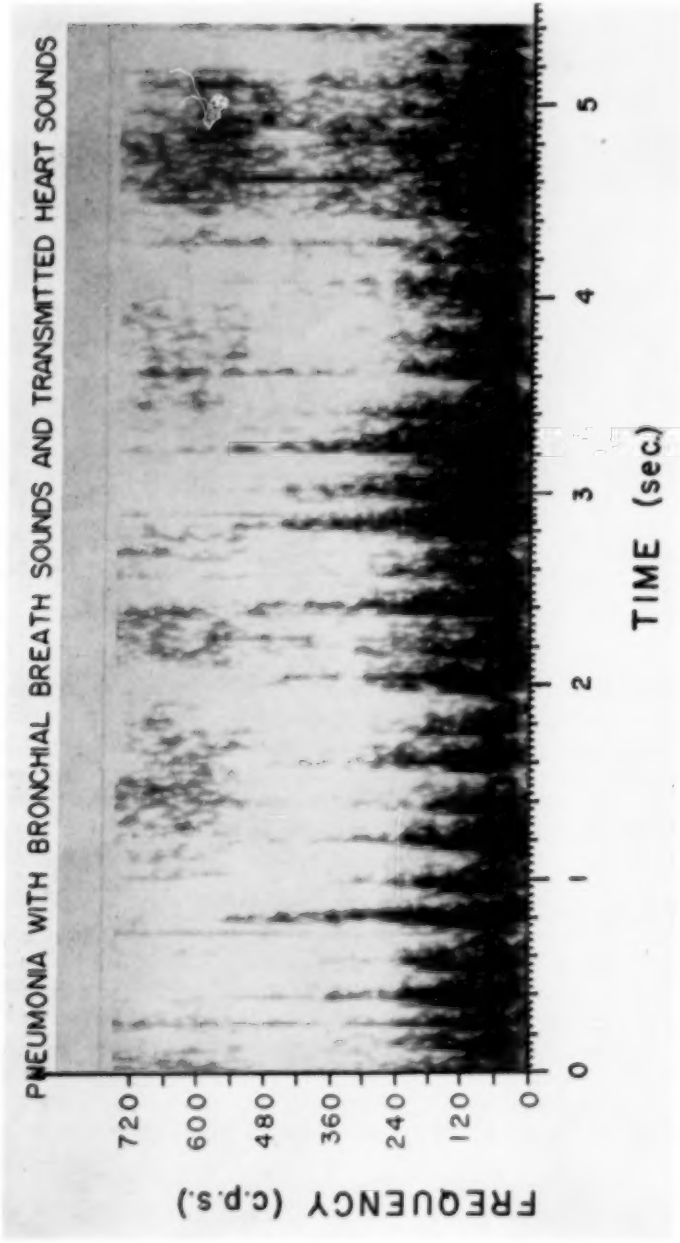

FIG. 7. Bronchial breath sounds and transmitted heart sounds in pneumonia of the left lower lobe.

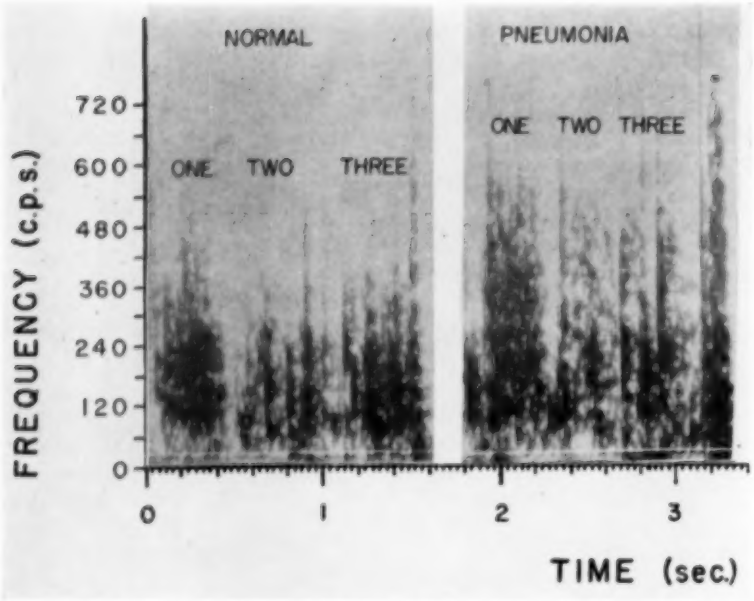

FIG. 8. Change in whispered sounds paralleling development of bronchovesicular breath sounds.

the character which gives the phenomenon the name whispered pectoriloquy (figure 9). The sounds are high-pitched with the same general frequency range as the inspiratory and expiratory sounds in bronchial breathing. (Different cases are illustrated in figures 7 and 9.) The genesis and significance of bronchial breath sounds and whispered pectoriloquy are identical. It is of interest to note in figure 9 that the sharp "t" sound of "two" (a so-called "plosive") is each time evident as a circumscribed sound indicated in the first instance by an arrow.

Changes in Spoken Voice Sounds

Analysis of the alterations in the spoken vowel sounds has yielded some of the most interesting results of the present studies. Contrary to what at first thought might seem to be the case, "E" is, on the average, a lower-pitched sound than "A." The analyses of the sounds "A" and "E" presented in figure 10 were made from recording of the sounds spoken directly into the microphone. Both sounds have conspicuous harmonics with characteristic curvature. In sound "A" the frequency span is greater, and the most intense (loudest) harmonics are in the vicinity of 360 c.p.s. as compared with 150 c.p.s. in the sound "E."

Over consolidated lung tissue, "E" spoken by the patient is likely to sound like "A"—the so-called "E-to-A" change. The analyses displayed in figures

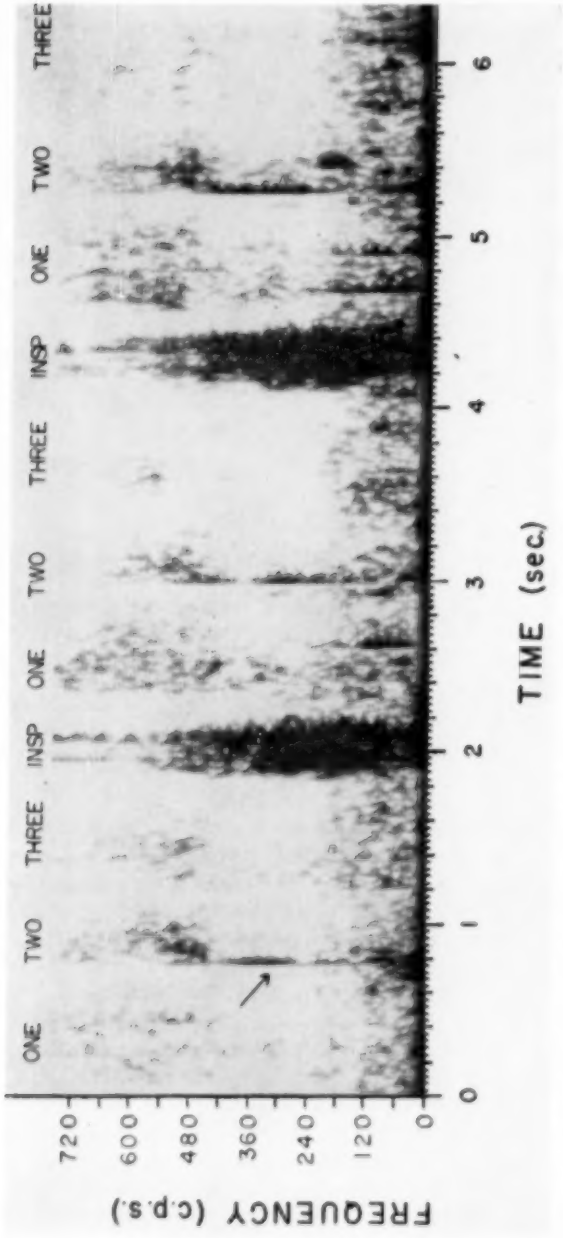

FIG. 9. Whispered pectoriloquy; changes in the whispered sounds paralleling the development of bronchial breath sounds.

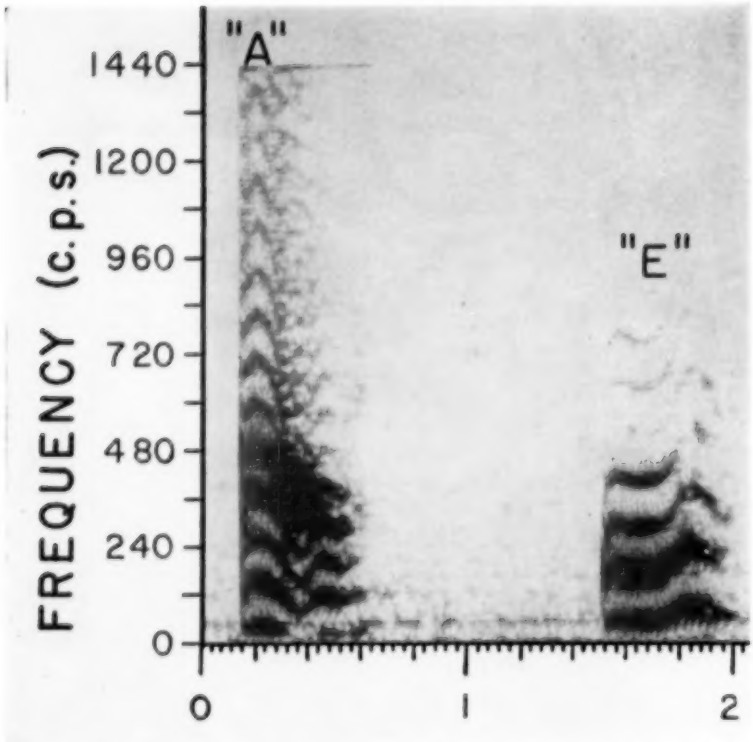

FIG. 10. The spectrogram of sounds "E" and "A" when spoken directly into the microphone.

11 and 12 demonstrate that this change is due to selective reinforcement (or at least selective increase in transmission) of certain ones of the higher harmonics of "E" and attenuation of lower ones. The result is a sound in which the harmonic pattern resembles more that of "A" than that of the parent sound "E."

This type of physical sign was probably discovered simultaneously (1922) by Shibley in China (22) and Fröschels and Stockert in Vienna (23). Shibley reported that the five vowel sounds "A, E, I, O, U" all became "A" or "Ah" in a localized area over fluid or consolidation and, indeed, also over a large goiter. He came upon this sign rather accidentally from having his Chinese patients say "i, er, san" (one, two, three). The word for "one" was pronounced "E" in his province; hence, the origin of the "E-to-A" sign.

Fröschels and Stockert (22) found that one vowel sound might be transformed into another with no particular predictability.

The patient from whom the recording analyzed in figure 13 was made had a pleural effusion on the right. The alteration is somewhat different from that seen in figures 11 and 12. Here "A", when detected at the upper level of the

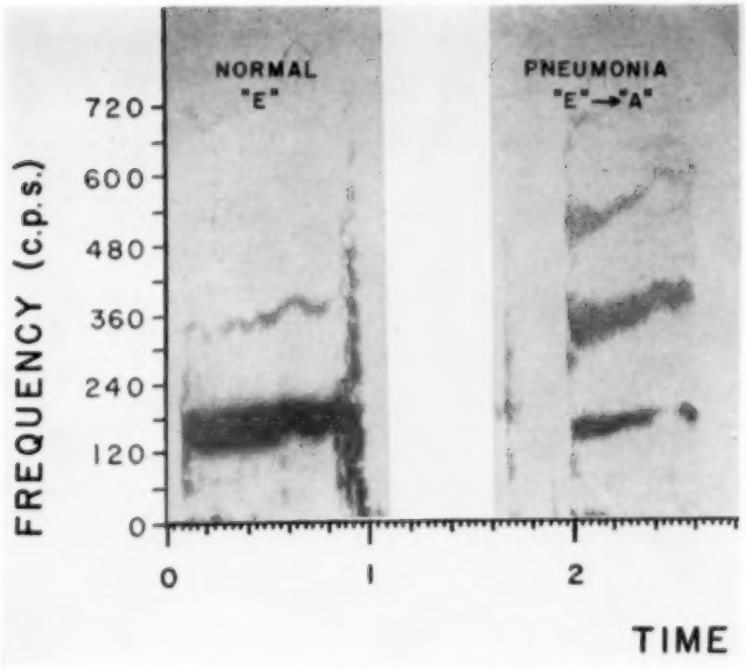

FIG. 11. "E to A change" over consolidated lung

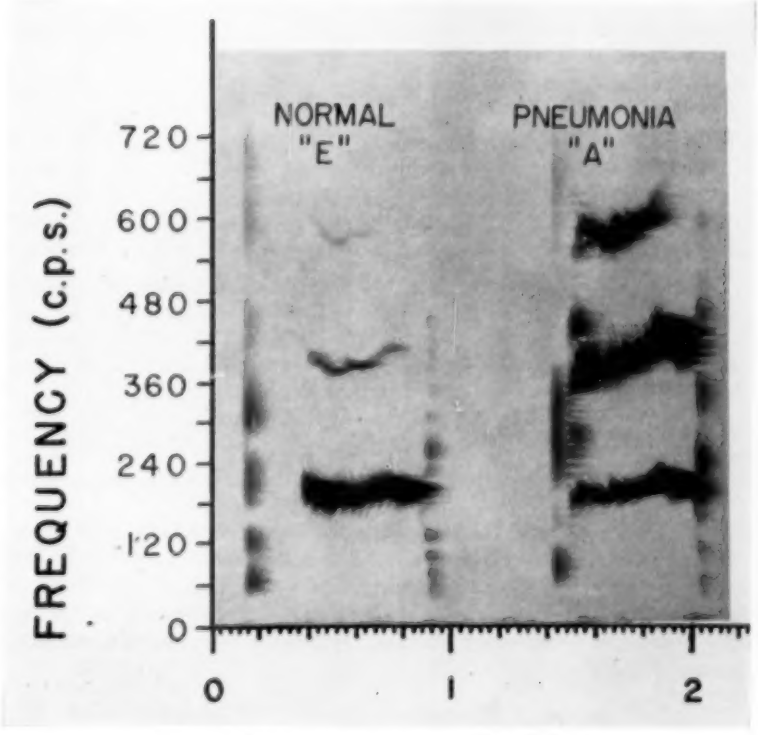

FIG. 12. "E to A change" over consolidated lung

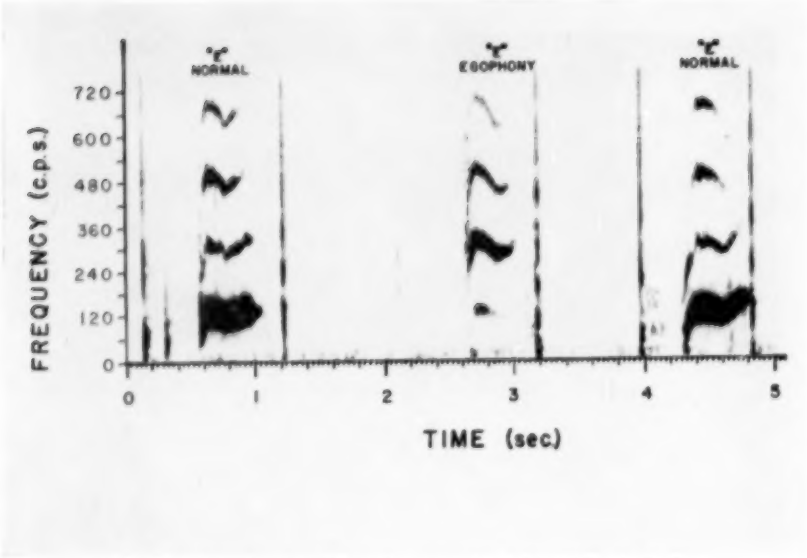

FIG. 13. Alteration in "E" at the upper level of a pleural effusion

fluid, sounded to the ear something like "ā" (as in "cat") with a bleating quality typical of egophony. The physical changes produced on the side of the fluid are (*1*) marked attenuation of the fundamental (at 160 c.p.s.), (*2*) reinforcement of the second harmonic (at 320 c.p.s.), and (*3*) attenuation of the fourth harmonic (at 640 c.p.s.). The behavior of the hemithorax which is partially filled with fluid is that of a "stiffer" pass-band filter: it has a higher "center frequency" and narrower frequency pass-band.

In the past much of speculative nature has been written about the physical origin of egophony. Norris and Landis (25) surveyed the theories in this manner:

> (*a*) Flattening of noncartilaginous bronchi by pressure of the effusion, so that they act like reeds (Laennec); (*b*) vibrations of the walls of small bronchi by actual collision—interruption of air currents (Wintrich); (*c*) the articulation of overtones—only the higher harmonics passing through the effusion (Stone); (*d*) vibrations passing through a thin layer of fluid.

Obviously, studies by sound spectrography lend support principally to the theory of Stone.

Rales

Rales, with the exception of the musical, sibilant ones produced in the bronchioles and occurring most dramatically in bronchial asthma, are circumscribed noises of limited duration, but considerable frequency span. It is possible by the method of sound spectrography to provide a physical definition for the extensive descriptive terminology for rales. Two extreme examples are illustrated in figures 14 and 15.

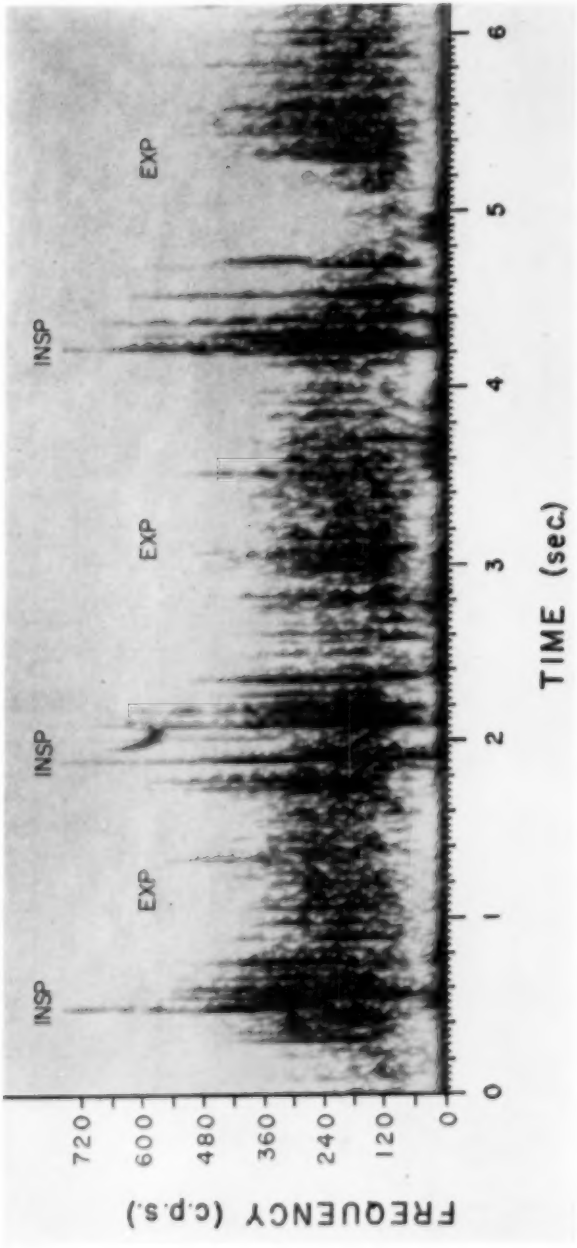

Fig. 14. Moist rales

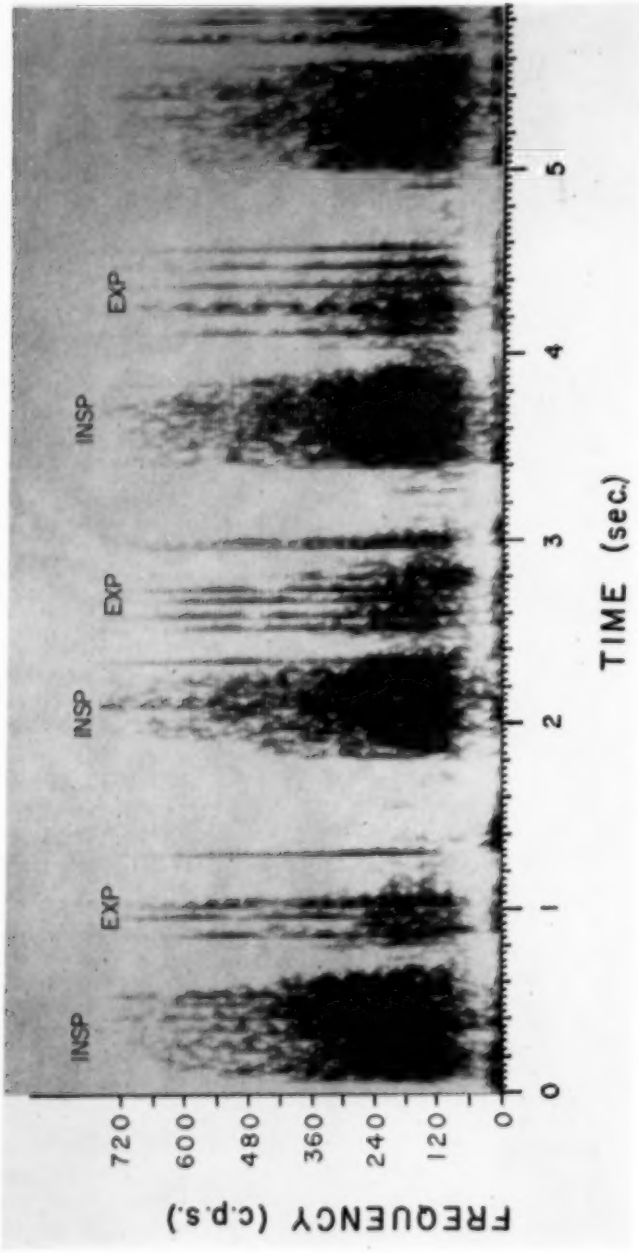

FIG. 15. Dry, crackling rales in expiration

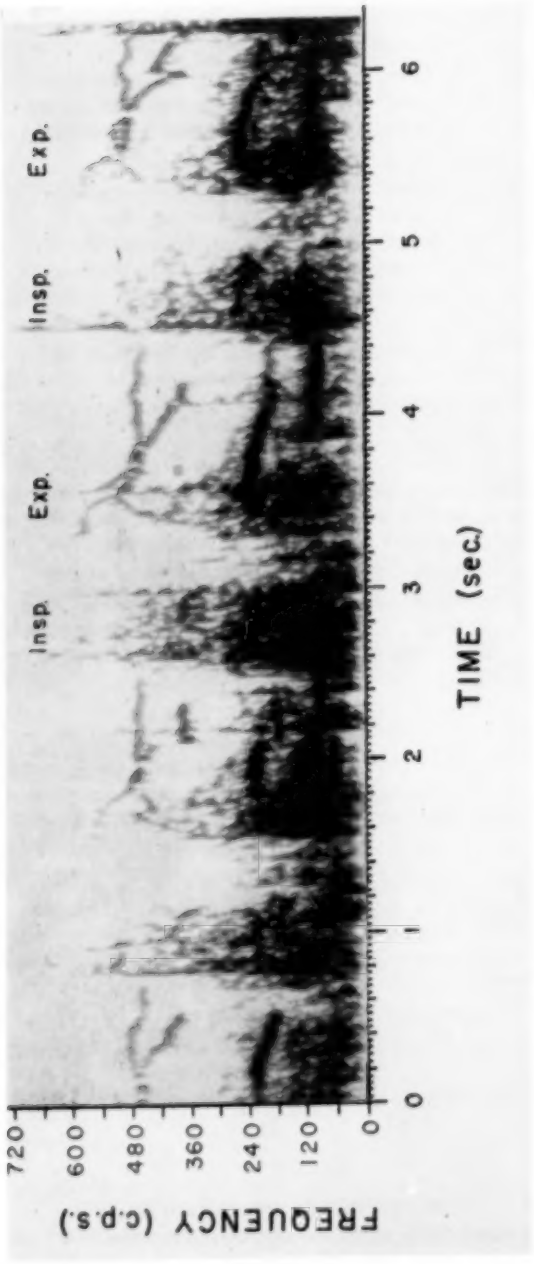

FIG. 16. Bronchial asthma

The patient illustrated in figure 14 had extensive bronchiectasis with superimposed pneumonitis. Numerous moist, bubbling rales are present in both inspiration and expiration. The breath sounds are bronchovesicular in character. In the second "inspiration," a wing-shaped harmonic at approximately 600 c.p.s. indicates a slight bronchiolar squeak which occurred at that point. Most of the rales in this instance display a fine harmonic pattern.

By way of contrast, the patient illustrated in figure 15 had dry, cracking rales limited to expiration. Pulmonary tuberculosis was present with predominant involvement of the right apex, from which region this recording was made. The harsh character of the inspiratory sound is evident. As to the rales, these sounds are characterized by a more diffuse uninterrupted frequency distribution than in the moist rales illustrated in figure 15. From other experience it is known that sharp sounds with this pattern are to the ear snapping and crackling in quality. In studying cardiovascular sound, for example, it is found that systolic clicks and the "opening snap" of mitral stenosis have this same general appearance in the spectrogram.

The musicality of the rales in bronchial asthma is the result of the presence of conspicuous harmonics (*see* figure 16; also figure 1). Expiration is prolonged and occupied by harmonics as demonstrated in the illustrations. Close scrutiny of the tracings reveals, as might be expected, that more than one "musical instrument" has been recorded. One produces a fundamental at 240 and a second harmonic at 480 cycles per second. This same sound generator also produces a fainter sound in inspiration. In two cycles, late in expiration a pair of harmonics are seen at 180 and 360 c.p.s. The two harmonics crossing at 480 c.p.s. almost certainly were generated in different bronchioles.

SUMMARY

The features of respiratory sound in health and disease have been studied by the method of sound spectrography. Most of the findings noted have been revealed or surmised previously by workers using other techniques. Nevertheless, by use of the method of sound spectrography, it has been possible to demonstrate the respiratory sounds in health and disease graphically to a greater extent than has previously been possible.

The physical differences which characterize dull, resonant, and tympanitic percussion notes, the several types of breath sounds, and moist and dry rales are demonstrated.

The change in whispered and spoken voice sounds in disease can be related to physical alterations in the conducting and filtering properties of the lung and its covering tissues.

The musicality of asthmatic rales results from the presence of conspicuous harmonics.

SUMARIO

La Base Acústica del Examen Torácico

Las caracteristicas de los ruidos respiratorios han sido estudiadas en la salud y la enfermedad por el método de la fonoespectrografia. La mayor parte de los hallazgos notados ya.

habían sido revelados o conjeturados antes por investigadores que usaron otras técnicas No obstante, mediante el uso del método fonoespectrográfico, ha sido posible observar gráficamente los ruidos respiratorios en la salud y la enfermedad en mayor grado de lo que había sido posible anteriormente.

Se muestran las diferencias físicas que caracterizan a las notas macizas, resonantes y timpánicas a la percusión, las varias formas de ruidos respiratorios y los estertores húmedos y secos.

El cambio observado en los ruidos de la voz cuchicheada y la hablada en la enfermedad pueden relacionarse con alteraciones físicas en las propiedades conductoras y filtradoras del pulmón y de sus tejidos de revestimiento.

La calidad musical de los estertores asmáticos proviene de la presencia de un notable tono armónico.

RESUME

La base acoustique de l'exploration pulmonaire

Les caractères des bruits respiratoires dans l'état de santé ou de maladie ont été étudiés par la spectrographie du son. La plupart des données enregistrées avaient été déjà révélées ou considérées possibles par d'autres chercheurs à l'aide d'autres techniques. Néanmoins l'emploi de la méthode de spectrographie du son a permis la démonstration graphique des bruits respiratoires dans l'état de santé ou de maladie à un degré plus grand qu'il n'avait été possible antérieurement.

Les différences physiques qui caractérisent les sons de percussion, matité, résonance, tympanisme, les différents types de murmures vésiculaires, les râles humides et secs, sont mis en évidence.

Les altération de la voix parlée ou chuchotée dans las conditions pathologiques peuvent être rapportées à des modifications physiques des propriétés de conduction et de filtration du poumon et des tissus qui le recouvrent.

Le caractére musical des râles asthmatiques est due à la présences d'harmoques prédominants.

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THE TREND OF TUBERCULOUS INFECTION AMONG SOME INDIAN TRIBES AND THE INFLUENCE OF BCG VACCINATION ON THE TUBERCULIN TEST[1]

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INTRODUCTION

Tuberculosis, unlike most infectious diseases, has not shown definite cyclic epidemics. Increases in the occurrence of the disease have usually been associated with the ravages of war or post-war conditions, with famine, or with marked economic depression. Although the statistics dealing with the mortality and morbidity from tuberculosis over a long period of time may be inaccurate because of faulty diagnosis or failure to report the cause of death, the fall of the death rate from this disease has been so great and continuous during the past century that it cannot be attributed solely to more accurate and complete diagnosis. More precise data indicating the rapid decline of tuberculosis are offered by the marked decrease of tuberculous meningitis, a type of tuberculosis which is usually diagnosed correctly. This form of tuberculosis decreased in New York City during the period from 1923 to 1949, inclusive, from a rate of 5.8 to 1.0 per 100,000, and in Philadelphia from a rate of 7.1 to 1.2 per 100,000.

Although the mortality and morbidity rates do furnish some measure of the extent and trend of tuberculosis, the tuberculin test is a more precise measure for determining the incidence and epidemiology of the disease.

There is a paucity of data dealing with the trend of tuberculous infection as determined by tuberculin surveys in a particular area over a period of years.

Harrington, Myers, and Levin (1) studied the trend of tuberculous infection in some rural areas in Minnesota. They found that the percentage of school children who reacted to tuberculin decreased between the years 1926 and 1936 from 47.3 to 17.9. The decline of tuberculous infection in children fifteen years of age and younger during the period 1930–1935 is reported by the Detroit Department of Health (2). It was found that, of children attending the tuberculosis clinic in Detroit, the percentage of persons who reacted to tuberculin declined from 54 in 1930 to 26 in 1935. In four counties in Minnesota, Jordon (3), using the intracutaneous test with 0.1 mg. of Saranac Lake Old Tuberculin (OT), found that 13.9 per cent of school children tested during the period 1930–1934 reacted while, in 1946–1947, only 2.7 per cent of the school children in the same counties reacted.

[1] From the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania; and the Branch of Health, Bureau of Indian Affairs, U. S. Department of the Interior, Washington, D. C.

PLAN OF INVESTIGATION

Description of Agencies

The different Indian tribes of the United States constitute relatively homogenous groups. They tend to remain in the same geographic areas and, in general, their economic condition is low. Housing is poor and houses are overcrowded, and the mortality and morbidity from tuberculosis are conspicuously higher than among the neighboring white population. These factors, coupled with the fact that a tuberculin survey had been conducted previously on certain Indian agencies, presented favorable conditions for determining whether or not the decline of tuberculosis which had been observed in some parts of the world, and especially in the United States, was evident among these groups of American Indians.

This investigation was conducted in five geographic areas of the United States and included six different tribes. They are the Pima Agency, Arizona, inhabited by the Pima Indians; the Wind River Agency, Wyoming, inhabited by the Shoshone and Arapaho tribes; the Rosebud Agency, South Dakota, inhabited by Sioux Indians; the San Carlos Agency, Arizona, inhabited by Apache Indians; and the Fort Apache Agency, Arizona, inhabited by the White Mountain Apaches. These agencies are widely scattered geographically and represent tribes which differ in their racial and linguistic stock, their degree of racial purity, their cultural backgrounds, and their dietary habits.

Pima Agency: The Pima Agency is located in the southwestern part of Arizona, approximately 40 miles southeast of Phoenix, Arizona. The Agency measures approximately 50 miles long and 6 to 15 miles wide. The Indians occupy 465,169 acres. In 1936 it was populated by 5,908 Pima Indians, almost all of whom were recorded as pure Indian stock. The population has gradually increased and, according to the 1950 census, numbered 7,562. The homes, which contain two to four small rooms, are one-story structures of adobe brick or adobe plastered on willows, while the floors usually consist of hard packed earth. In general, overcrowding prevails, although during the warmer months some members of the household sleep in a ramada adjacent to the house. Sewage is disposed on the surface, and outhouses are uncommon.

The Pima Indian diet is high in carbohydrates and low in protein. Their diet formerly consisted of flour, corn, squash, mesquite beans, cactus fruit, and some small game. At the present time they continue on a high carbohydrate diet, but they no longer grind their wheat on the metate and in general buy much the same variety of food as the white population, with beef excepted. Of this they buy relatively small amounts. In recent years, visits to adjacent towns have been facilitated by public buses which pass through the Agency.

Formerly these Indians maintained themselves by farming, cotton picking, and fattening of cattle. In recent years an increasing number work for wages on ranches adjacent to the Agency, on the railroad, and in industrial plants. Among these Indians, tuberculosis and diabetes are the most common diseases. Coccidioidin sensitivity was found in 83.6 per cent of 1,419 of the population tested in 1938, but clinical cases of this disease are rare (4). During 1936–1937, 354 children of pre-school and school age received BCG vaccine, but these persons were not included in the present tuberculin survey.

Wind River Agency: The Wind River Agency is located in the north central part of Wyoming, approximately 15 miles north of Lander, Wyoming. The Agency is approximately 55 miles long and 40 miles wide. The Indians occupy an area of 829,440 acres. The Agency is inhabited by the Shoshone and Arapaho tribes, which differ not only linguistically but also in racial stock, physical appearance, and cultural pattern. The Shoshones

are of Shoshonean stock and belong linguistically to the Uto-Aztecan group. The Shoshone population has increased from 1,122 in 1936 to 1,527 in 1950. Approximately 67 per cent of this tribe were listed as mixed Indian stock on the 1936 tribal rolls. These Indians occupy the northwestern part of the Agency. Social contacts between the two tribes are limited, and intermarriage is rare. Tuberculosis as well as other infectious diseases are less common among the Shoshones than among the Arapahos.

The Arapahos, who are of Algonquin stock, were placed on this Agency by the U. S. Government in 1877 and have continued to occupy the southeastern part of the Agency. In 1936 the census showed an enrollment of 1,125 Arapahos and, according to the 1950 census, this number had increased to 1,624. Approximately 30 per cent were listed on the tribal rolls of 1936 as being of mixed Indian stock. The incidence of both pulmonary and extrapulmonary tuberculosis and of rheumatic fever and other diseases associated with streptococcal infection is conspicuously higher among the Arapahos than among the Shoshones, despite the fact that living conditions are about the same for members of both tribes. The frame or log homes of both tribes are usually small and overcrowded, although in recent years there has been some improvement in housing conditions. The income of both tribes is derived from farming and cattle raising. In recent years additional income has come from royalties from oil wells located on the Agency. The diet of both tribes in general is high in protein. They consume large amounts of beef and relatively small amounts of vegetables and cereals. In 1936, 110 Shoshones and 118 Arapahos received BCG vaccine, but only 3 of these persons were inadvertently included in the present tuberculin survey.

Rosebud Agency: The Rosebud Agency, located in the south central part of South Dakota approximately 15 miles southwest of the town of Mission, measures 139 miles long and 60 miles wide. This Agency is populated by the Brule branch of the Dakota Sioux Indians, who occupy 1,116,509 acres of the Agency. In 1940, the population numbered 6,042 Indians, 58 per cent of whom were listed as being of mixed Indian stock and, according to the 1950 census, the population had increased to 7,035. The economic condition of these Indians has long been poor, due in part to periods of drought. Farming and stock-raising have in the past been their main sources of income. During the period after World War II, many migrated to adjacent areas to help in the harvest, and others settled in nearby cities where they work for wages. The size and structure of the homes vary within wide limits. Some of the better ones are well-built frame or log houses, but many Indians continue to live in overcrowded and overheated "A" type tents. Their diet is high in protein and in general consists of beef, corn, potatoes, and beans. This group of Indians has a high rate of both pulmonary and extrapulmonary forms of tuberculosis. Involvement of the cervical lymph nodes and joints with tuberculosis, particularly the knee joint, is not uncommon. In 1937, BCG vaccine was administered to 260 children of school age. This group of vaccinated persons was not tested during the present tuberculin survey. Nine children vaccinated at birth in 1939–1940 were included, inadvertently, in the 1954 study.

San Carlos Agency: The San Carlos Agency is located south of the east central area of Arizona, approximately 15 miles east of Globe. It is populated by Apache Indians, who represent the most southerly extension of the Athapascan family. The Agency covers 1,622,484 acres of land. The Indian population, which numbered 2,983 in 1939, had increased to 3,858 by 1952. Approximately 95 per cent of these Indians are listed as being of pure Indian stock.

Until recently the majority of the Indians lived in "wickiups." These are conical shaped shelters nine to ten feet high made of brush placed over poles and covered with canvas or other material. A central opening at the top permits the escape of smoke and helps to

ventilate the place. Due to recently improved economic conditions resulting from the raising and sale of cattle, the wickiup has been replaced to a great extent by small frame houses. Outhouses are few, and sewage is disposed on the surface. The farm land is divided into two-acre plots which serve as subsistence gardens, while the main source of income is from cattle raising.

In the past the diet was chiefly corn, beans, squash, and large game, but in recent years the diet is much the same as that of the neighboring white population. In 1940–1941 (4), sensitivity to coccidioidin was found in approximately 98 per cent of 543 children of school age tested, although clinical manifestation of this disease was not observed. The incidence of tuberculosis has been high among this tribe. During the 1940–1941 studies, BCG vaccine was not administered to these Indians.

Fort Apache Agency: The Fort Apache Agency is located in the east central part of Arizona approximately 20 miles south of McNary, Arizona. It is separated, on its southern boundary, from the San Carlos Agency by the deep canyon of the Salt River. The Agency covers an area of approximately 73 by 56 miles. It is populated by members of the White Mountain Apache band, who occupy 1,664,782 acres of the Agency. The population which numbered 2,987 in 1941 had increased to 3,825 in 1953. Nearly all of these Indians are of pure Indian stock. Many of them continue to live in wickiups, although an increasing number of small frame houses are being built. Outhouses are scarce, and sewage is thrown on the surface.

The economy of these Indians before World War II, which was based on small truck gardening and on cattle raising, has changed in recent years to an increasing wage economy. Many of the Indians are now employed in the lumbering industry in the areas adjacent to the Agency. The diet is high in carbohydrates such as tortillas and quick bread. The mortality and morbidity from tuberculosis continue to be high among these Fort Apache Indians. Although they live approximately 50 miles north of the San Carlos Agency, where the incidence of coccidioidin sensitivity is high, only 15 per cent of 527 children of school age tested with coccidioidin in 1940–1941 on this Agency reacted (4) and, of these, many gave a history of having visited the San Carlos Agency. BCG vaccine was not administered in 1940–1941 to the Indians of this reservation.

1954 Tuberculin Survey of School Children

To determine the trend of tuberculous infection among Indian children of school age attending either the Indian Service, public, or mission schools, a tuberculin survey was conducted during March and April, 1954, on those selected agencies where tuberculin surveys had been conducted previously, and the results of the two surveys were compared. All but 12 of the persons who had received BCG vaccine in 1936–1937 as well as those who served as controls at that time were excluded from the present study since, with but few exceptions, they were no longer of school age.

In order that the results of this tuberculin survey should be compared with the initial survey, the same procedure and the same type of purified protein derivative tuberculin (PPD) were used.

Procedure

As a sample of the PPD used in the original study was no longer available, Mr. H. J. Henderson, of the Henry Phipps Institute, prepared a lot of PPD by adding trichloracetic acid (5) to crude tuberculin prepared from cultures grown on a synthetic medium. This

crude tuberculin was kindly furnished by the Bureau of Animal Industry, Washington, D. C. The sample of PPD used in this study assayed 14 per cent nitrogen while the original sample of PPD, kindly furnished by Dr. Florence Seibert, Henry Phipps Institute, assayed 14.1 per cent nitrogen (6). As with the original PPD, the dry powder was first dissolved in a weak solution of sodium hydroxide and the stock solution was made up in a concentration of 10 mg. per ml. The required dilute tuberculin for testing was prepared daily by adding sterile buffered physiologic saline to the concentrated PPD. The diluted tuberculin was used only on the day of preparation.

As in the initial survey, the tuberculin test was carried out by injecting 0.00002 mg. of PPD, in a volume of 0.1 ml., as superficially as possible into the skin over the volar surface of the forearm. The site of injection was examined forty-eight hours later. When a definite local inflammatory reaction was present, the extent of edema was determined by measuring the palpable edema in two dimensions and estimating the thickness of edema. When the local reaction to the initial dose of 0.00002 mg. was negative, doubtful, or slight, a second injection of 0.005 mg. of PPD in a volume of 0.1 ml. was administered in the opposite arm. The site of the second injection was observed, with few exceptions, forty-eight hours later. The degree of reaction was recorded as 1-plus, 2-plus, 3-plus, 4-plus, doubtful, or negative on the basis of the measurement of the area of edema (7).

The tuberculin testing was carried out on a large percentage of Indian children enrolled either in the federal, public, or mission schools on the agencies or in the public schools adjacent to the agencies. Of the 1,192 children of school age enrolled in 1953–1954 in the federal or mission schools on the Pima Agency, Arizona, and in one public high school adjacent to the Agency, 1,070 (89.7 per cent) were tested. On the Wind River Agency, Wyoming, 221 (70.9 per cent) of the 312 Shoshone children and 305 (66.4 per cent) of the 459 Arapahos enrolled in the various schools on the Agency and in a public high school adjacent to the Agency were tested. Of the 930 children enrolled in the schools on the Rosebud Agency, South Dakota, in 1953–1954, as well as in the public schools of the nearby town of Mission, South Dakota, 812 (87.3 per cent) were tested. On the San Carlos Agency, 744 (83.3 per cent) of the 893 enrolled in the federal and mission schools on the Agency and in one public high school adjacent to the Agency were tested. At Fort Apache, 835 children attending a federal boarding school, federal day schools, a public school, and two mission schools were tested, although the school census showed only 821 enrolled for 1953–1954.

OBSERVATIONS

In the course of the present study a total of 3,987 Indian children ranging in age from five to nineteen years, inclusive, received an initial dose of 0.00002 mg. of PPD tuberculin. Of this group, 945 (23.7 per cent) reacted. The 3,042 persons who failed to react to this dose of PPD were injected with 0.005 mg. of PPD and, of the 2,747 persons who returned for a final reading, 722 (26.3 per cent) reacted.

In the initial surveys of 1936–1937 and 1940–1941, a total of 5,754 children, ranging in age from five to nineteen years, were tested with 0.00002 mg. of PPD. Of those tested, 3,036 (52.8 per cent) gave an unequivocal, definite reaction. Because of this high level of sensitivity and because none of the population had at that time received BCG vaccine, it may be assumed that the reaction to 0.00002 mg. of PPD was the specific result of a spontaneous infection with virulent tubercle bacilli.

Early in the course of the 1954 study it was noted that, among the older children, a small but significant number of slight, doubtful, or atypical reactions followed the injection of 0.00002 mg. of PPD and that a comparatively large

number of the older children who did not react to 0.00002 mg. did react to 0.005 mg. of PPD. As a low level of tuberculin hypersensitivity and doubtful or atypical reactions have been observed over a period of years more frequently among BCG-vaccinated persons than among unvaccinated persons (8, 9), it was suspected that some of these children might have received BCG vaccine at some time previously.

It was learned that in 1949 the Office of Indian Affairs, in cooperation with the U.S. Public Health Service, had conducted an extensive tuberculin-testing and BCG vaccination program on different agencies in the United States, including the agencies of this study, and that the records were on file in Washington, D. C. After the present progam of tuberculin testing was completed, the relevant files of the U. S. Public Health Service, Washington, D. C., were made available for study through the cooperation of Dr. Carroll E. Palmer and Mr. Lawrence W. Shaw. The files included the names of those who had received BCG vaccine in 1949, the names of those who were tuberculin-negative in 1949 but who were not vaccinated, the names of those whose tuberculin test was not completed, and the names of those who were tuberculin-positive at that time. This information was not available at the local agencies.

A cross-checking of the lists of the present survey with the Public Health Service records showed that no person in the present five- to nine-year age group has been included in the 1949 Office of Indian Affairs–U. S. Public Health Service project. Of the ten- to nineteen-year age group, 1,485 had been included in the 1949 study in one category or another, and 842 of these had received BCG vaccine. These 842 persons comprised 31.3 per cent of the 2,693 persons who make up the ten- to nineteen-year age group of our 1954 study.

The five- to nine-year age group tested in 1954 was the one group in which no child had received BCG vaccine in 1949. It is comparable therefore to the five- to nine-year age group tested in the initial studies of 1936–1937 and 1940–1941. The percentage of the five- to nine-year age group which reacted to 0.00002 mg. of PPD decreased from 31.3 per cent for the six agencies or tribes in the initial studies to 11.7 per cent in the 1954 studies (table 1 and figure 1).

The results of the 1954 survey for the older age groups are not entirely comparable with the results of the initial studies, since 29.5 per cent of the ten- to fourteen-year age group and 35.3 per cent of the fifteen- to nineteen-year age group had received BCG vaccine in 1949, which presumably resulted in a low level of tuberculin hypersensitivity in a high percentage of cases.

The percentage of the ten- to fourteen- and fifteen- to nineteen-year age groups which reacted to 0.00002 mg. of PPD in the initial studies as compared with the percentage which reacted in 1954 is presented in five-year age periods in table 1 and figure 1. Despite the inclusion in these calculations of those persons who received BCG vaccine in 1949, the percentage of reactors among the ten- to fourteen-year age group to 0.00002 mg. of PPD decreased from an average of 57.0 for those tested in the initial study to an average of 22.8 in 1954. Of the fifteen- to nineteen-year age group, the percentage of those persons who reacted to 0.00002 mg. of PPD in the initial tests was 81.0, while, in 1954, only 44.6 per cent of this age group reacted. This marked decrease in the percentage of

TABLE 1

COMPARISON OF PERCENTAGE OF TUBERCULIN REACTIONS IN INITIAL SURVEYS AND IN 1954 BY DOSE OF TUBERCULIN (PPD), AGENCY, AND IN FIVE-YEAR PERIODS

5-9 Years

Agency or Tribe	Year Initial Test	Initial Tests					1954 Tests				
		Number Tested	Percentage Positive to 0.00002 Mg. of PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive	Number Tested	Percentage Positive to 0.00002 Mg. of PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive
Pima	1936–1937	638	16.8	531	7.9	23.4	407	3.9	354	4.2	7.9
Shoshone	1936	103	20.4	82	4.4	23.9	78	9.0	64	1.6	10.5
Arapaho	1936	122	38.5	75	6.6	42.6	103	13.6	81	2.5	15.8
Rosebud	1937	575	41.9	334	6.3	45.6	216	30.1	135	8.9	36.3
San Carlos	1940–1941	256	18.7	208	13.0	29.3	251	3.6	225	4.9	8.3
Fort Apache	1940–1941	473	45.4	258	6.9	49.2	239	17.2	177	2.8	19.5
Total		2,167	31.3	1,488	7.9	36.7	1,294	11.7	1,036	4.4	15.6

10-14 Years

Agency or Tribe	Year Initial Test	Initial Tests					1954 Tests				
		Number Tested	Percentage Positive to 0.00002 of Mg. PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive	Number Tested	Percentage Positive to 0.00002 Mg. of PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive
Pima	1936–1937	520	37.7	324	13.6	46.2	469	9.0	388	37.6	43.2
Shoshone	1936	92	52.2	44	9.1	56.5	108	16.7	78	25.6	38.0
Arapaho	1936	115	68.7	36	5.6	70.5	144	26.4	93	30.1	48.6
Rosebud	1937	726	62.1	275	10.2	66.0	370	44.1	186	33.9	63.1
San Carlos	1940–1941	290	40.7	172	21.5	53.4	363	11.3	294	33.3	40.8
Fort Apache	1940–1941	544	73.9	132	15.9	78.0	420	30.0	272	33.8	53.7
Total		2,287	57.0	983	13.8	62.9	1,874	22.8	1,311	34.1	49.1

15-19 Years

Agency or Tribe	Year Initial Test	Initial Tests					1954 Tests				
		Number Tested	Percentage Positive to 0.00002 Mg. of PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive	Number Tested	Percentage Positive to 0.00002 Mg. of PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive
Pima	1936–1937	257	68.9	80	30.0	78.2	194	25.8	130	60.8	70.9
Shoshone	1936	59	76.3	14	28.6	83.1	35	25.7	21	38.1	54.0
Arapaho	1936	82	85.4	12	—	85.4	58	43.1	26	38.5	65.0
Rosebud	1937	414	82.9	72	9.7	84.6	226	65.9	71	56.3	85.1
San Carlos	1940–1941	124	71.8	35	28.6	79.9	130	28.5	80	57.5	69.6
Fort Apache	1940–1941	364	90.7	34	44.1	94.8	176	54.0	72	63.9	83.4
Total		1,300	81.0	247	24.3	85.6	819	44.6	400	57.3	76.3

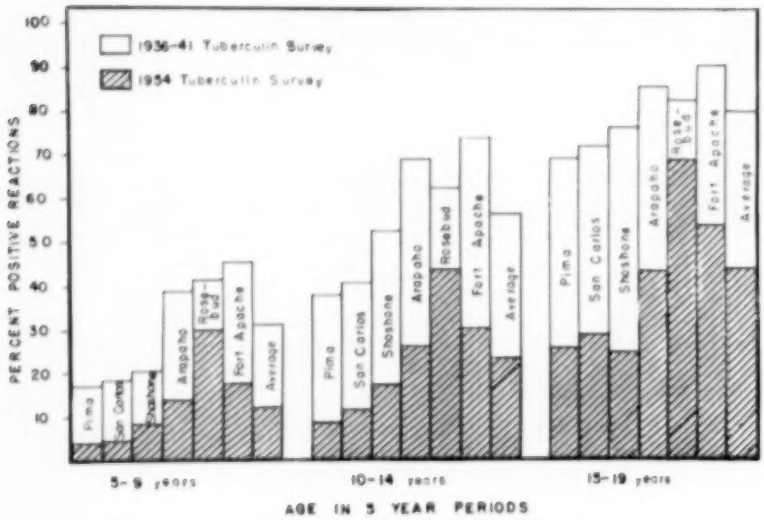

FIG. 1. Percentage of positive reactions to 0.00002 mg. of PPD in 1936–1941 and in 1954.

those who reacted to 0.00002 mg. of PPD in 1954 over that observed in the initial study is the most convincing evidence of the decrease of tuberculous infection among the Indian groups studied.

The percentage of persons showing a high level of tuberculin hypersensitivity varied widely among the different tribes. These variations were unrelated to the geographic location of the tribes. It will be noted from table 1 and figure 1 that the smallest percentage of persons who reacted to 0.00002 mg. of PPD occurred both in the initial survey and in the 1954 survey among the Pima tribe, Pima Agency, Arizona; the Apache tribe, San Carlos Agency, Arizona; and the Shoshone tribe, Wind River Agency, Wyoming. The highest percentage of reactors in both surveys occurred among the Arapaho tribe, Wind River Agency, Wyoming; the Sioux tribe, Rosebud Agency, South Dakota; and the White Mountain Apache, Fort Apache Agency, Arizona. The higher percentage of reactors among the latter three tribes is correlated with the higher morbidity and mortality from tuberculosis in these areas.

It will be noted in table 1 and figure 2 that the percentage of persons who failed to react to 0.00002 mg. but reacted to 0.005 mg. of PPD among the five- to nine-year age group decreased from 7.9 in the initial studies to 4.4 in the 1954 study. In contrast, in the ten- to fourteen- and fifteen- to nineteen-year age groups a greater percentage among those failing to react to 0.00002 mg. reacted in 1954 to the second dose of tuberculin than in the initial studies. The explanation for this increase in the percentage of those who reacted to 0.005 mg. of PPD among the two older age groups lies in the fact, as previously stated, that 29.5 and 35.3 per cent of the ten- to fourteen- and fifteen- to nineteen-year

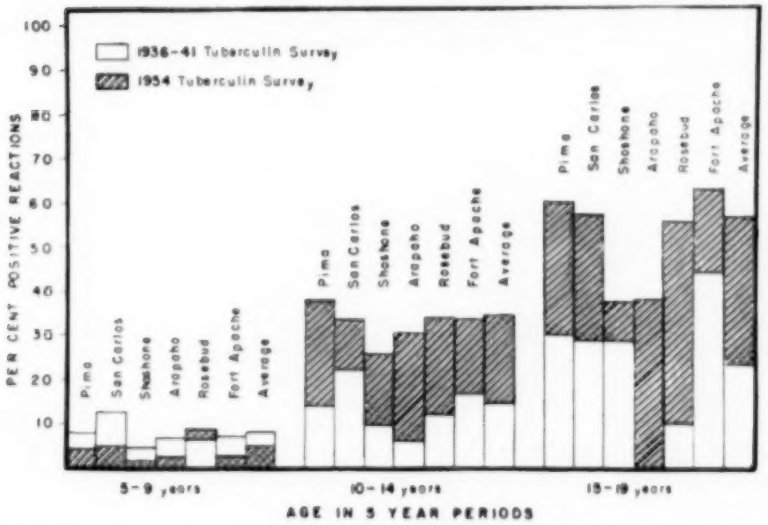

FIG. 2. Percentage of positive reactions to 0.005 mg. of PPD in 1936-1941 and in 1954.

age groups, respectively, had received BCG vaccine in 1949 and subsequently developed a post-vaccinal low level of tuberculin hypersensitivity.

The possibility that the high percentage of reactions to 0.005 mg. of PPD noted in 1954 may be nonspecific due to differences in geographic location of the groups tested, as described by Palmer, Ferebee, and Petersen (10), was considered. The data presented in table 1 for both the initial studies and for the 1954 study fail to show any relationship between the percentage of those who reacted to the second dose of PPD and the geographic location of those tested. A further possibility is that, among the two older age groups, a smaller percentage of those tested in 1954 reacted to 0.00002 mg. of PPD than in the initial studies, thereby making available a larger percentage of persons to be tested with 0.005 mg. of PPD. However, this explanation is not valid since the data presented in table 2, which exclude those tested in 1949, indicate that, despite the larger percentage available for testing with 0.005 mg. of PPD, approximately the same percentage reacted to 0.005 mg. of PPD as in the initial studies.

The total percentage of those who reacted to PPD tuberculin as presented in table 1 is corrected for the 295 persons of the 1954 study who failed to return for examination. It will be noted from this table that the most marked reduction in the total percentage of persons who reacted to PPD in 1954 over that observed in the initial studies occurred among the five- to nine-year age group and that the reduction in the two older age groups was less marked, since it was masked in part by the inclusion of those who had received BCG vaccine in 1949.

A more precise measure of the difference in the percentage of persons who reacted to tuberculin among those tested in the initial studies as compared with

TABLE 2

COMPARISON OF PERCENTAGE OF TUBERCULIN REACTIONS (PPD) AMONG UNSELECTED AND NON-BCG-VACCINATED NORTH AMERICAN INDIANS IN 1936–1941 AND IN 1954 BY FIVE-YEAR AGE PERIODS AND DOSE OF TUBERCULIN

Year Tested	5–9 Years					10–14 Years					15–19 Years				
	Number Tested	Percentage Positive to 0.0002 Mg. of PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive	Number Tested	Percentage Positive to 0.0002 Mg. of PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive	Number Tested	Percentage Positive to 0.0002 Mg. of PPD	Number Tested	Percentage Positive to 0.005 Mg. of PPD	Total Percentage Positive
1936–1941	2,167	31.3	1,488	7.9	36.7	2,287	57.0	983	13.8	63.0	1,300	81.0	247	24.3	85.6
1954	1,294	11.7	1,036	4.4	15.6	936	23.5	647	15.6	35.4	272	43.0	136	30.9	60.6

the 1954 study can be derived by excluding from the calculations of the 1954 study all persons who were tuberculin tested during the survey conducted jointly by the Office of Indian Affairs and the U. S. Public Health Service in 1949. At that time some of those who failed to react to tuberculin received BCG vaccine, while others were not vaccinated but served as controls. Furthermore, among the ten- to fourteen- and fifteen- to nineteen-year age groups tested at that time were some who were tuberculin-positive and others who did not complete the test.

The results of the tuberculin survey of the unselected population in the initial studies are compared in table 2 with the results of the tuberculin tests in 1954 on the unselected population only, excluding all those who had been in any of the groups tuberculin tested in 1949. The results presented in this table show a significant reduction in tuberculous infection in the age group tested as indicated by the marked decrease in the percentage of those with a high level of tuberculin hypersensitivity between the initial studies and 1954.

The results observed following the injection of 0.005 mg. of PPD in 1954 also show a marked reduction in the percentage of reactors among the five-to nine-year age groups from that noted in the initial studies. The percentage of reactors at this dosage level in the ten- to fourteen- and fifteen- to nineteen-year old groups in 1954 was slightly higher than that noted in the initial studies.

Comment on the influence of BCG vaccination on epidemiologic studies: The development of a positive tuberculin reaction to a small dose of tuberculin among those who have not received BCG vaccine or any other specific immunizing agent can be accepted as evidence of natural infection with virulent tubercle bacilli. However, among BCG-vaccinated persons, the development of tuberculin hypersensitivity can be attributed to the BCG vaccination, to superimposed infection, or to both.

It has been claimed (11) that the use of BCG vaccine, followed as it is in a high percentage of cases by a change of the tuberculin reaction from negative to positive, may interfere with the use of the tuberculin reaction as a measure of

tuberculous infection. This objection is valid within certain limits and deserves serious consideration, especially since the decrease of tuberculous infection in recent years has greatly increased the importance of the tuberculin test. The present writers have been aware of this complication for a long time and have attempted to establish criteria which would help in distinguishing the tuberculin sensitivity due to BCG vaccination from that due to natural infection with virulent tubercle bacilli.

Previous studies (8, 9) have shown that the parenteral administration of BCG vaccine to tuberculin-negative persons results in tuberculin hypersensitivity differing quantitatively and qualitatively in most instances from that resulting from natural infection. The present writers have found among BCG-vaccinated persons that the level of tuberculin hypersensitivity is generally low, with only a small percentage reacting to the smaller amounts of Old Tuberculin, PPD, or PPD-S, and a large percentage reacting to the larger amounts of the different tuberculins. The local reaction, observed forty-eight hours following the injection of the different tuberculins in BCG-vaccinated persons, is characterized by a diffuse deep red, faint red, or reddish purple color associated with edema, which varies in degree from definite, sharply defined, visible edema to slight or barely palpable diffuse edema. With increasing years following the BCG vaccination, if there is no superimposed infection, the level of tuberculin hypersensitivity decreases and an increasing number convert to doubtful or negative.

In contrast, among the unvaccinated who have undergone a natural infection, as well as among the BCG-vaccinated who have been infected with virulent tubercle bacilli, the level of tuberculin hypersensitivity is usually high. The local reaction when observed forty-eight to seventy-two hours following the intracutaneous injection of the smaller dose of any one of the different tuberculins is usually characterized by a well-marked local inflammatory reaction. The redness and edema are conspicuous, and the edema is readily palpable. Frequently induration is noted in the center of the edematous area. In the more hypersensitive persons lymphangitis, vesiculation, necrosis, or small petechial hemorrhages may be noted at the site of injection. Among those with a high level of tuberculin hypersensitivity, only a small percentage convert to negative with passing years. Thus, Dahlstrom (12) found that 2.8 per cent of 2,130 persons who reacted to either 0.01 mg. of OT or to 0.00002 mg. of PPD reverted to negative, while 60.3 per cent of 360 persons who reacted only to 0.1 or 1.0 mg. of OT or 0.005 mg. of PPD converted to negative.

When BCG-vaccinated persons undergo infection with virulent tubercle bacilli, a sharp increase is noted in the level of tuberculin hypersensitivity which is indistinguishable from that observed among unvaccinated persons who undergo natural infection. Aronson and Aronson (13) observed that among BCG-vaccinated persons the appearance of pulmonary lesions having the roentgenographic characteristics of tuberculosis was associated with an increase in the level of tuberculin hypersensitivity.

The present writers' observations indicate that, although the differences between the tuberculin reaction following natural infection and that following

TABLE 3

COMPARISON OF TUBERCULIN REACTION IN 1954 BY DOSE OF PPD AND DEGREE OF REACTION IN UNVACCINATED AND BCG-VACCINATED PERSONS

Attained Age in Five-Year Periods	Group	Number Tested	Doubtful to 0.00002 Mg.		Positive to 0.00002 Mg.		Percentage of Positive Reactors by Degree				Number Tested	Doubtful to 0.005 Mg.		Positive to 0.005 Mg.		Percentage of Positive Reactors by Degree			
			Number	Per cent	Number	Per cent	1+	2+	3+	4+		Number	Per cent	Number	Per cent	1+	2+	3+	4+
10-14	Unvaccinated	1,321	27	2.0	383	29.0	1.3	12.6	14.9	0.23	842	118	14.0	144	17.1	2.3	10.6	3.2	—
10-14	Vaccinated	553	40	7.2	45	8.1	1.4	4.8	2.2	—	468	88	18.8	303	64.7	8.3	45.7	10.7	—
15-19	Unvaccinated	530	18	3.4	305	57.5	4.7	30.4	22.3	0.19	196	22	11.2	71	36.2	6.6	15.8	13.8	—
15-19	Vaccinated	289	28	9.7	60	20.8	5.9	11.1	3.8	—	201	29	14.2	158	77.5	5.4	46.6	25.5	—

BCG vaccination are in general quite distinguishable, they do not categorically divide one type of reaction from the other. Among unvaccinated persons with a low level of tuberculin hypersensitivity, the tuberculin reaction may be indistinguishable from that resulting from BCG vaccination.

On the basis of the present writers' observations in this and in previous studies, it is believed that BCG vaccination does not seriously interfere with studies dealing with the epidemiology of tuberculosis by means of the tuberculin test, since so few vaccinated persons who have not undergone a superimposed infection react to the small dose of 0.00002 mg. of PPD or to 0.01 mg. of Old Tuberculin.

Comparison of vaccinated and unvaccinated persons with respect to 1954 tuberculin reaction: The results of the 1954 tuberculin tests among unvaccinated persons and among those who received BCG vaccine in 1949 are presented in table 3. The unvaccinated group tested in 1954 consisted of those persons who, when tested in 1949, failed to react to tuberculin and were not vaccinated; those who reacted to tuberculin; those whose reactions were not read; and those who were not tuberculin tested. The vaccinated group consisted of those persons who failed to react to the tuberculin test in 1949 and who received an intracutaneous injection of BCG vaccine at that time.

It may be seen in this table that differences in the level of tuberculin hypersensitivity between the unvaccinated and the BCG-vaccinated persons were indicated not only by the higher percentage of the unvaccinated who reacted to 0.00002 mg. of PPD but also by the significantly higher percentage of persons who gave strong reactions to this dose of PPD.

Among the BCG-vaccinated persons, doubtful or atypical reactions were noted more frequently than among the unvaccinated, and weak 1-plus reactions to 0.00002 mg. of PPD were noted with equal frequency in both the unvaccinated and vaccinated of the same age group. The small percentage of the BCG-vaccinated persons who showed the more intense 3-plus reactions represent, in all probability, those who had undergone a superimposed infection with virulent tubercle bacilli.

An indication of the low level of tuberculin hypersensitivity induced by BCG vaccination is shown not only by the small percentage of persons who reacted to 0.00002 mg. of PPD but by the high percentage who reacted to 0.005 mg. of PPD (table 3). Among the ten- to fourteen-year age group, a somewhat higher percentage of the vaccinated than unvaccinated gave a doubtful or 1-plus reaction to 0.005 mg. of PPD. Among the fifteen- to nineteen-year-old group, a somewhat higher percentage of the vaccinated gave a doubtful reaction to this dose of PPD, while the 1-plus reaction occurred with almost equal frequency for both groups. For each age group a significantly higher percentage of the vaccinated than unvaccinated showed a 2- or 3-plus reaction to 0.005 mg. of PPD. Many of the unvaccinated and BCG-vaccinated persons who gave a 3-plus reaction were those who had given a doubtful or atypical reaction to the initial dose of 0.00002 mg. of PPD.

In the joint study of the Office of Indian Affairs and U. S. Public Health Service conducted in 1949, the maximum dose of PPD used was 0.0002 mg., and those who failed to react to that dose of PPD were classified as tuberculin-negative. In the present study the second or final dose of PPD was increased to 0.005 mg., in order to make the dose comparable with that used in the initial studies of 1936–1941, at which time those who failed to give a definite reaction to 0.005 mg. of PPD were considered tuberculin-negative.

Environmental factors: The present project, limited as it was to the same six Indian tribes whose tuberculous infection rate had been previously studied by means of the tuberculin test, offered an opportunity not only to determine the trend of this disease but to consider some of the factors responsible for the trend. In contrast to the non-Indian population, such environmental factors as housing, dietary habits, economic conditions, and cultural pattern are relatively uniform for the members of each of the six tribes. As a consequence, there are fewer variables requiring consideration in an attempt to evaluate the cause of changes in the rate of tuberculous infection.

The present study has revealed that a significant decline in tuberculous infection has occurred in children of school age between 1936–1941 and 1954. For the first time since 1936, when the controlled study on tuberculous infection and the effects of BCG vaccination was instituted, some classrooms on these Indian agencies were found without a single reactor to tuberculin.

Although the time spent on each agency was insufficient for an exhaustive study of living conditions, sufficient data were furnished by the superintendent of each agency and his staff which, coupled with the observations of the writers, permitted some general conclusions whether changes in cultural patterns, housing and economic conditions, as well as BCG vaccination, had influenced the trend of tuberculous infection.

A comparison of housing conditions in 1954 with those observed in the past showed no significant reduction in overcrowding. The number of new houses has not been commensurate with the increase of population.

Cash income among the Indians has increased greatly since the original studies. It must be realized, however, that incomes among these Indians in

the period before World War II were very low and that at the present time their income is approximately one-half that of the non-Indians in adjacent areas. Among the Pima, White Mountain Apaches, and Rosebud Sioux, the economy has changed from an agricultural to a wage economy. Improvement in economic conditions on some of the agencies is reflected in the increased purchase of household appliances, farm equipment, automobiles, and better clothing.

Dietary habits have undergone definite changes since 1936, especially among the Indians of the southwest. The younger Indians, many of them trained in home economics, no longer use the native plants or grind their grain on the metate. The local stores carry much the same variety of groceries, clothes, and other items as are found in any well-stocked country store. Local storekeepers state that the type of purchases made by Indians is comparable with that made by non-Indians.

Among the customs which are rapidly disappearing are the use of the cradle board for infants and the sling used for carrying older children on the mother's back. With automobiles rapidly replacing the springless horse-drawn wagon, there is greater mobility, and visits to outlying towns are more frequent.

Among the important tangible factors which have contributed to the control, not only of tuberculosis but of other diseases, is the Indian Service field nurse. She has gained the confidence of the families in her area and, in the school room, in the clinic, and in their homes, has taught them the value of hygiene and preventive medicine.

* * *

Effect of BCG Vaccination on Infection Rate

There are numerous experimental and observed data indicating that resistance to tuberculosis can be increased by immunization with viable avirulent tubercle bacilli. There are almost no data, however, on what effect specific immunization may have on the subsequent trend of tuberculous infection in the community.

In 1936–1937, BCG vaccine was administered intracutaneously to approximately one half of a group of tuberculin-negative pre-school and school-age children living on the Pima, Fort Washakie, and Rosebud Agencies in Arizona, Wyoming, and South Dakota, respectively. At the same time the remaining half of the tuberculin-negative children received physiologic saline and served as controls. Both vaccinated and control subjects were followed for nine to eleven years by means of annual tuberculin tests and roentgenographic examinations of the chest. The results of this controlled study indicated that the cumulative mortality from tuberculosis after thirteen to fifteen years of observation (14) and the morbidity as estimated from annual roentgenographic examination of the chest for nine to eleven years (15) were both significantly lower among the BCG-vaccinated than among the controls.

In 1940–1941 the school children living on the San Carlos and Fort Apache Agencies in Arizona were tuberculin tested, but BCG vaccine was not administered at that time to the tuberculin-negative persons. However, in the

joint program of the Office of Indian Affairs and the U. S. Public Health Service conducted on all of the above-mentioned agencies in 1949, BCG vaccine was administered to a certain percentage of those who failed to react to 0.0002 mg. of PPD.

It will be noted from table 1 that the decrease in the percentage of those who reacted to 0.00002 mg. of PPD in all age groups was unrelated to the use of BCG vaccine in 1936–1937. Among those living on the San Carlos or Fort Apache Agencies in Arizona, where BCG vaccine was not used until 1949, the decrease in the percentage of those who reacted to 0.00002 mg. of PPD in 1954 was as marked as that observed in the other agencies, where BCG vaccine was first used in 1936–1937 and again in 1949.

DISCUSSION

Although the present study presents objective evidence of a definite decrease of tuberculous infection among the Indian school children studied, it is difficult, if not impossible, to determine the specific factors responsible for this decrease.

There has been less exposure in recent years to manifest cases of tuberculosis. This has been brought about not only by better facilities for diagnosis and earlier hospitalization but also by educating the public to the dangers and mode of spread of tuberculosis. Accuracy of diagnosis has been improved through the availability of competent radiological consultants and installation of roentgenographic equipment and laboratory facilities in most of the Indian Service hospitals.

Housing continues to be inadequate and overcrowding has probably increased with a growing population. Cash incomes are larger, but there is no exact information concerning expenditures. Dietary habits have remained unchanged or show a continuing change from traditional native foods to those used by the neighboring white population.

The data presented in this study do not provide direct evidence that BCG vaccination was a contributing factor in reducing tuberculous infection. Unfortunately the period between the initial tuberculin tests and the 1954 survey was four to five years longer on the agencies where BCG vaccine was used in 1936–1937 than on the San Carlos and Fort Apache Agencies, where BCG vaccine was not used in 1936–1937 and where tuberculin tests were first made in 1940–1941. Nevertheless, one cannot ignore the evidence from the previous study (14) that the cumulative mortality rate from tuberculosis following thirteen to fifteen years of observation was 3.32 and 0.56 per thousand person-years of observation for the unvaccinated controls and BCG-vaccinated persons, respectively. Likewise, in the earlier studies (15), pulmonary lesions having the roentgenographic characteristics of tuberculosis were found in 16.4 per cent of the control subjects and in 4.1 per cent of the vaccinated subjects who were followed for nine to eleven years by annual roentgenographic examination of the chest. This reduction in the mortality and morbidity from tuberculosis in the vaccinated group undoubtedly reduced the number of foci of tuberculous infection in these areas.

SUMMARY

The trend of tuberculous infection among school-age children of six Indian tribes was determined by comparing the percentage of those who reacted to 0.00002 mg. and 0.005 mg. of PPD tuberculin in studies conducted in 1936–1937 and in 1940–1941 with the percentage of comparable age groups among the same tribes who reacted in 1954.

A marked reduction in the percentage of persons who reacted to 0.00002 mg. of PPD was observed among the 3,987 children tested in 1954 in comparison with the percentage of the 5,754 children of comparable age who reacted to the same dose of PPD in the initial studies.

A significant reduction in the percentage of those who did not react to 0.00002 mg. but reacted to 0.005 mg. of PPD in 1954 was noted in the five- to nine-year age group, none of whom had received BCG vaccine.

A significant increase in the percentage of persons who did not react to 0.00002 mg. but reacted to 0.005 mg. of PPD in 1954 over that noted in the initial studies was found in the ten- to fourteen- and fifteen- to nineteen-year age groups. This increase presumably resulted from the inclusion in the study of some of those who received BCG vaccine in 1949.

When the character of the groups which were tuberculin tested in the initial studies and in 1954 is made comparable by excluding all those tested in 1949, the percentage of reactors in the ten- to fourteen- and fifteen- to nineteen-year age groups, who reacted only to 0.005 mg. of PPD, is approximately the same in the 1954 tests as in the initial tests. The reduction in the percentage of those who reacted to 0.00002 mg. of PPD in 1954 over the initial studies is approximately the same, whether those tested in 1949 are excluded or not.

The total percentage of reactors in 1954 to both doses of PPD decreased markedly for the five- to nine- and ten- to fourteen-year age groups and to a lesser degree for the fifteen- to nineteen-year age group.

Among the two older groups which had received BCG vaccine in 1949, fewer reacted to 0.00002 mg. of PPD than among the unvaccinated, while a significantly higher percentage of those vaccinated in 1949 reacted only to 0.005 mg. of PPD than of the unvaccinated.

The tuberculin reaction following the intracutaneous administration of BCG vaccine generally differs quantitatively and qualitatively from that following natural infection with virulent tubercle bacilli.

SUMARIO

La Tendencia de la Infección Tuberculosa en Algunas Tribus Indias y el Influjo de la Vacunación BCG sobre la Reacción a la Tuberculina

Se determinó la tendencia de la infección tuberculosa entre los niños de edad escolar de seis tribus indias comparando el porcentaje de los que reaccionaron a 0.00002 mg. y 0.005 mg. de tuberculina PPD en estudios ejecutados en 1936–1937 y en 1940–1941 con el porcentaje en los grupos de edad comparable de las mismas tribus que reaccionaron en 1954.

Observóse una reducción notable en el porcentaje de personas que reaccionaron a 0.00002 mg. de PPD entre los 3,987 niños comprobados en 1954, comparado con el porcentaje entre los 5,754 niños de edad comparable que reaccionaron a la misma dosis de PPD en los estudios iniciales.

Notóse una importante reducción en el porcentaje de los que no reaccionaron a 0.00002 mg., pero sí a 0.005 mg., de PPD en 1954, en el grupo de cinco a nueve años de edad, ningún niño del cual había recibido vacuna BCG.

Descubrióse un aumento significativo en el porcentaje de personas que no reaccionaron a 0.00002 mg., pero sí a 0.005 mg., de PPD en 1954, sobre el notado en los estudios iniciales en los grupos de diez a catorce y quince a diecinueve años de edad. Este aumento provenía presuntamente de la inclusión en el estudio de algunos de los que recibieron vacuna BCG en 1948.

Cuando la naturaleza de los grupos comprobados con tuberculina en los estudios iniciales y en 1954 se hace comparable por la exclusión de todos los comprobados en 1949, el porcentaje de reactores en los grupos de diez a catorce y quince a diecinueve años de edad, que no reaccionaron más que a 0.005 mg. de PPD, es aproximadamente idéntico en las pruebas de 1954 y en las pruebas iniciales. La reducción en el porcentaje de los que reaccionaron a 0.00002 mg. de PPD en 1954, comparado con el de los estudios iniciales, es aproximadamente idéntica, ya se excluya o no a los comprobados en 1949.

El porcentaje total de reactores a ambas dosis de PPD en 1954 disminuyó notablemente para los grupos de cinco a nueve y diez a catorce años de edad y en menor proporción para el grupo de quince a diecinueve años de edad.

Entre los dos grupos de mayor edad que habían recibido vacuna en 1949, menos reaccionaron a 0.00002 mg. de PPD que entre los no vacunados, mientras que, en 1949, un porcentaje significativamenta mayor de los vacunados que de los no vacunados reaccionó solamente a 0.005 mg. de PPD.

La reacción a la tuberculina después de la administración intracutánea de vacuna BCG discrepa en genera cuantitativa y cualitativamente de la consecutiva de la consecutiva a la infección natural con bacilos tuberculosos virulentos.

RESUME

La tendance de l'infection tuberculeuse parmi certaines tribus indienne et l'influence de la vaccination par le BCG sur réaction à la tuberculine

La tendance de l'infection tuberculeuse parmi les écoliers de six tribus indiennes a été déterminée en comparant le pourcentage des sujets réagissant à 0,00002 mg et à 0,005 mg de tuberculine PPD dans les études pratiquées en 1936–1937 et en 1940–1941 avec le pourcentage de réactions positives obtenu en 1954 dans des groupes d'âge comparable parmi ces mêmes tribus.

Une réduction importante du pourcentage de sujets qui réagissaient à 0,00002 mg de PPD a été observé chez les 3.987 enfants soumis aux tests en 1954, par comparaison avec le pourcentage obtenu chez 5.754 enfants d'âge comparable réagissant à cette même dose de PPD dans les études initiales.

Une réduction significative du pourcentage de ceux qui ne réagissaient pas à 0,00002 mg., mais qui réagissaient à 0,005 mg de PPD en 1954, a été observée dans le groupe 5–9 ans, dont aucun n'avait reçu le BCG.

Le pourcentage de sujets ne réagissant pas à 0,00002 mg de PPD, mais qui réagissaient à 0,005 de PPD en 1954, a montré un accroissement significatif par rapport aux chiffres des études initiales dans les groupes 10–14 ans et 15–19 ans. Cet accroissement para't imputable à l'inclusion dans cette étude de quelques sujets ayant reçu le vaccin BCG en 1949.

Quand le type des groupes ayant été testés à la tuberculine dans les études initiales et en 1954 a été rendu comparable par l'exclusion de tous les sujets testés en 1949; le pourcentage des sujets réagissant seulement à 0,005 mg de PPD dans les groupes 10–14 ans et 15–19 ans est approximativement le même dans les tests de 1954 que dans les tests initiaux. Par rapport aux études initiales, la réduction du pourcentage de ceux qui réagissaient à 0,00002 mg de PPD en 1954 est approximativement le même, avec ou sans exclusion des sujets testés en 1954.

Le pourcentage total des sujets à réaction positive de 1954 pour les deux doses de PPD

s'est considérablement abaissé pour les groupes 5–9 ans et 10–14 ans, et à un degré moindre pour le groupe 15–19 ans.

Parmi les deux groupes plus âgés ayant reçu le BCG en 1949, le nombre de ceux qui réagissaient à 0,00002 mg de PPD était inférieur à celui obtenu chez les non vaccinés, alors que le pourcentage des sujets vaccinés en 1949 ne réagissant qu'à 0,005 mg était accru de façon significative, par rapport aux non vaccinés.

La réaction à la tuberculine, à la suite de l'administration intracutanée de BCG diffère généralement quantitativement et qualitativement de celle qui succède a l'infection naturelle par les BK virulents.

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MYCOBACTERIUM FORTUITUM[1]

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INTRODUCTION

The acid-fast bacillus described in this paper was isolated from the cervical lymph nodes of a woman in Malta. The cultural characters of the bacillus conform to those of *Mycobacterium fortuitum*, a species described by Cruz in 1938.

OBSERVATIONS

Clinical Record

A girl, aged 19, working on a farm in Malta, was suffering pain from a carious molar tooth during the Christmas season of 1953. She dug at the tooth with a new pair of scissors and removed part of the tooth and some carious material. This maneuver caused what she described as serious bleeding, which she stopped by pressing a handkerchief over the bleeding spot. Approximately fifteen days later she noticed a swelling in the right side of the neck and, a few days later, another swelling under the chin. Both swellings were said to be painless, but as they increased in size she attended the Outpatient department of St. Luke's Hospital, Malta, on January 18, 1954.

Her past history and family history showed nothing of medical interest. She was one of a family of eight, all of whom are alive and well. There was no history of tuberculosis in the family. On examination, the only abnormalities found were: (*1*) carious second molar teeth in the lower jaw on both sides; (*2*) a mass of enlarged lymph nodes under the middle third of the right sterno-mastoid muscle; (*3*) an enlarged submental node; and (*4*) a slight degree of anemia with a hemoglobin value of 88 per cent. There was no leukocytosis, and no enlarged mediastinal nodes or signs of infiltration were present in a roentgenogram of the chest.

On January 25, 1954, and again on February 1, some pus was aspirated from the cervical nodes through a wide-bore needle. Microscopic examination of the pus showed a few acid-fast bacilli, but no other bacteria. Acid-fast bacilli were the only bacteria to grow on Löwenstein-Jensen medium, Difco tryptose agar, and blood agar. On February 8 the abscess in the cervical nodes was incised and drained. The patient was treated with 1 gm. of chloramphenicol daily for five days.

On February 15 the patient was admitted to the surgical wards of St. Luke's Hospital under the care of Professor A. J. Craig. The abscess had re-formed and there was now evidence of pus in the submental nodes. Hemoglobin was 72 per cent; leukocytes were 11,700 per cu. mm; polymorphonuclears, 6,552; lymphocytes, 4,797; monocytes, 351. The erythrocyte sedimentation rate was 70 mm. in the first hour. A roentgenogram of the chest showed some increase in bronchovascular markings, possible slight enlargement of the nodes at the left hilum, but no evidence of infiltration. The Mantoux test was negative with dilutions of 1:10,000 and 1:1,000 Old Tuberculin (OT), but gave an area of edema 10 by 10 mm. with 1:100. The patient was treated with streptomycin, 1 gm. daily; para-aminosalicylic acid (PAS), 12 gm. daily; isoniazid, 300 mg. daily; liver extract, iron, and vitamins. On March 17 a blood culture was sterile.

While the patient was in the hospital, there was a slight rise in temperature on Febru-

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ary 18. This persisted until March 3, when it rose to 100.8°F. During the next three days the temperature reached 104°F. and then declined. On March 7 it was 102.4°F. Since then it has been normal or subnormal. PAS was discontinued on March 5 and penicillin, 500,000 units, was given daily for six days.

The patient's condition improved and the erythrocyte sedimentation rate dropped to 37 mm. in the first hour on March 25. On the next day the patient's submental node was surgically excised for histologic examination. The patient was discharged on April 28 with a minimal lymphatic enlargement.

Acid-fast bacilli were cultured from the purulent exudate on five occasions in addition to the two already mentioned. The last positive culture was from pus obtained on May 23, 1954. A sample of pus obtained on March 27 did not reveal any acid-fast bacilli on culture.

Histologic Appearance of Submental Node

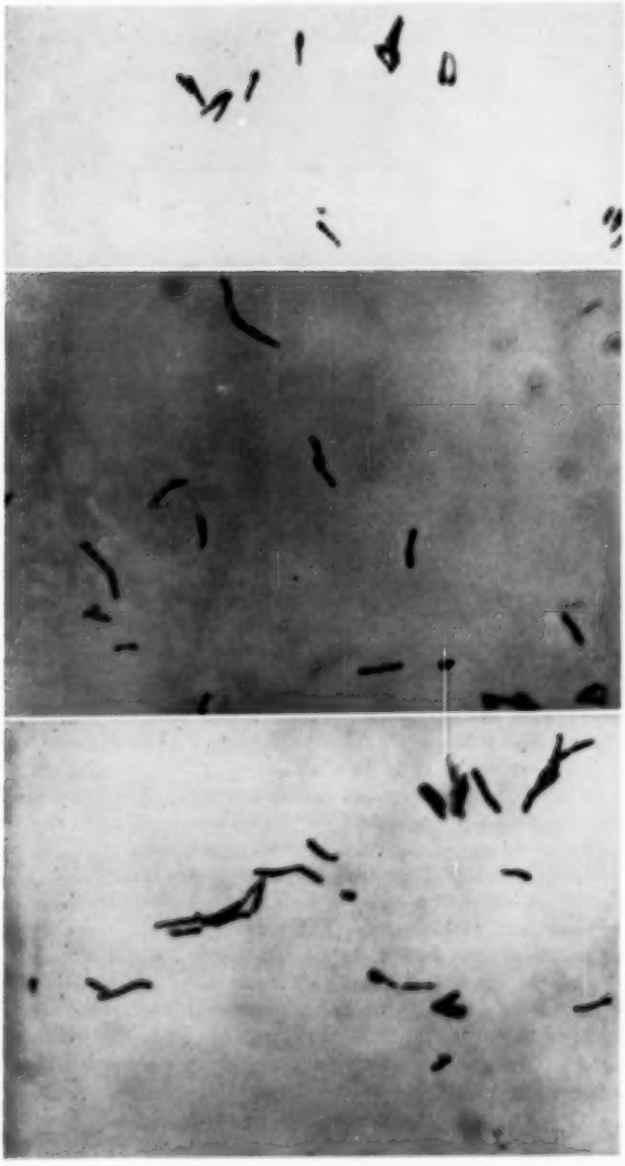
A report from Dr. A. H. T. Robb-Smith states:

> The sections show that the specimen consisted of fatty connective tissue, with some striated muscle fibers in which there is a granulomatous area. It is impossible to determine whether or not the granulomata arose in lymphoid tissue; no structure characteristic of a lymph node can now be identified.
>
> The granulomatous area is characterized by epithelioid necrotic congeries surrounded by a capsule of young formed fibrous tissue in which degenerating muscle fibers are included in places. Within the fibrous capsule is a zone of lymphocytes and plasma cells which surround epithelioid congeries which tend to coalesce and, in the coalescent areas, necrosis has taken place.
>
> At first glance the appearance of the epithelioid granulomata is very suggestive of that seen in a tuberculous infection and, although that cannot be excluded on morphological grounds, yet there are certain features that are not commonly seen in tuberculous infection; the principal difference is that a high proportion of the epithelioid cells show lipoid vacuolation with differentiation to a fat carrying macrophage; further, the tissue necrosis is not a true caseation.
>
> Sections stained by Ziehl-Neelsen, Gram, and silver impregnation failed to reveal any microorganisms.
>
> *Diagnosis: Epithelioid Granulomata.*

Bacteriologic Studies

A series of bacteriologic studies were performed and the general characteristics of the microorganism can be summarized as follows:

> The bacillus is variable both in length and thickness. An unusual feature is the presence of swellings, which have the morphologic appearance of spores (figures 1 to 3). These are seen, both in stained smears of pus from the patient and, more frequently, in stained films of cultures, particularly those grown on Löwenstein-Jensen medium and glucose agar. These swellings may be situated terminally, centrally, or lying free. According to Lack and Tanner (1), such swellings in mycobacteria are nuclei and are apparent because of the unfavorable medium in which the bacilli find themselves.
>
> The bacillus is Gram-positive and acid-fast. The staining with Gram's stain is entirely comparable with that of *Mycobacterium tuberculosis*. With the Ziehl-Neelsen stain, the bacillus resists decolorization with 25 per cent sulfuric acid for ten minutes followed by hydrochloric acid alcohol for one minute. Partial decoloriza-



FIGS. 1–3. *M. fortuitum*. Films from fourteen-day culture on Löwenstein-Jensen medium. (Ziehl-Neelsen stain, × 900).

TABLE 1

GROWTH CHARACTERISTICS OF MYCOBACTERIUM FORTUITUM IN VARIOUS TYPES OF MEDIA

Medium	Growth Characteristics
Löwenstein-Jensen	Smooth, glistening, pale straw-colored colonies at first; later becoming matt. The edge is irregular and the whole colony like coral. There is a slightly fetid smell.
Dorset's egg with or without glycerin.	Round, glistening, heaped-up, pale straw-colored colonies with worm-cast growth in the center. Slightly fetid smell.
5% Serum agar	Round, glistening, colorless colonies with heaped-up center.
5% Glycerin agar	As on serum agar.
5% Glucose agar	Large, spreading, colorless colonies glistening at first and later matt, with an irregular edge and raised leaf-like veins. Slightly fetid smell.
Lemco agar	Glistening, colorless colonies with an irregular edge and heaped-up center.
Oleic-acid–albumin agar	At first, round, hemispherical, smooth colonies with a slightly fetid smell. There is no cording. After 7 days' incubation the colonies are crateriform.
Horse blood agar	No hemolysis.
5% Glycerin broth	Pellicle growth, very easily dislodged and falling to the bottom of the tube. No turbidity of the medium.
5% Glucose broth	
Lemco broth	
CCY Broth	
Dubos Tween® 80	Uniform turbidity
Christensen's medium	Hydrolyses urea in 48 hours

tion takes place if hydrochloric acid alcohol is used for ten minutes. Complete decolorization follows if 25 per cent sulfuric acid is applied for four hours.

The bacillus grows readily both at 37° and at 22°C. in liquid media and on solid media. In primary culture, growth was seen in both liquid and solid media in seven days. In subculture there was good growth in three days. The growth characteristics in those media which have been used are shown in table 1.

The colonial appearance is not markedly different on various solid media with the exception of 5 per cent glucose agar (figures 4, 5). Here the colony spreads rapidly and soon becomes matt. There is, however, no difference in colonial appearance on oleic-acid–albumin agar when suspensions are seeded from the matt-spreading colonies of glucose agar or from the glistening colonies of other solid media. Suspensions of bacilli from Löwenstein-Jensen medium, Lemco broth, and glucose agar were tested for heat resistance at temperatures of 50°, 60°, and 80°C. The bacilli from Löwenstein-Jensen and Lemco broth were four days old; the suspension from glucose agar was forty-five days old. The results are shown in table 2.

Drug susceptibility: The bacillus was found to be resistant to 16.3 units of penicillin per ml. in liquid Tween®-albumin medium, and also to 2.5 mg. of sulfadiazine. The susceptibility to streptomycin and isoniazid is shown in table 3.

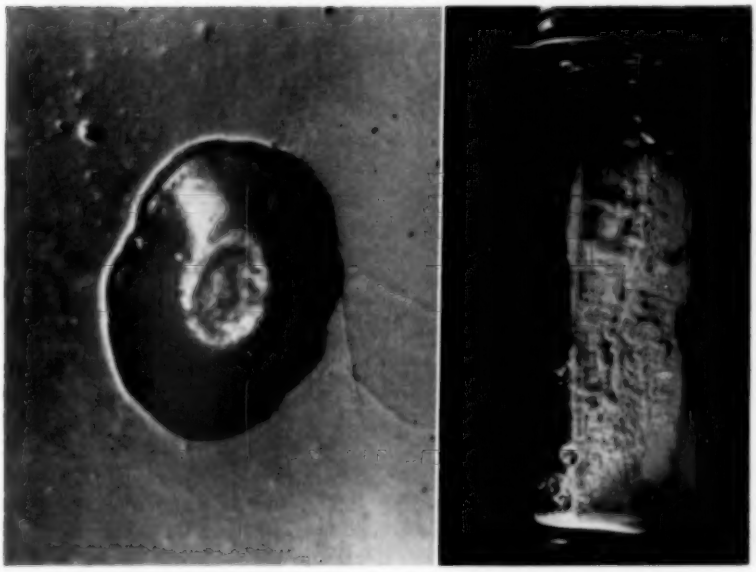


FIG. 4 (left) *M. fortuitum*. Seven-day colony on oleic acid–albumin agar.
FIG. 5. (right) *M. fortuitum*. Seven-day culture on 5 per cent glucose agar.

TABLE 2

HEAT RESISTANCE OF SUSPENSIONS OF BACILLI

Temperature	Time	Löwenstein-Jensen 4-Day Culture	Lemco Broth 4-Day Culture	Glucose Agar 45-Day Culture
37°C.	–	++	++	++
50°C.	1 minute	++	++	
	5 minutes	++	++	
	15 minutes	++	++	
60°C.	1 minute	++	++	
	5 minutes	+	+	
	15 minutes	–	+	
80°C.	1 minute	–	–	+
	5 minutes	–	–	–
	15 minutes	–	–	–

TABLE 3

SUSCEPTIBILITY OF M. FORTUITUM TO STREPTOMYCIN AND ISONIAZID

Streptomycin					Isoniazid				
Units per Ml.	Days				Units per Ml.	Days			
	1	2	3	4		1	2	3	4
100.0	–	–	–	–	100.0	–	–	–	+
50.0	–	–	–	–	50.0	–	–	–	+
25.0	–	–	–	±	25.0	–	–	±	+
12.5	–	–	–	±	12.5	–	–	+	+
6.25	+	+	+	+	6.25	–	±	+	+
3.125	+	+	+	+	3.125	+	+	+	+
Control	+	+	+	+	Control	+	+	+	+

Pathogenicity

Rabbit: The intravenous injection of 0.2 mg., moist weight, of bacilli into a rabbit failed to produce any obvious disease. When the rabbit was killed sixty days after the injection, the only abnormality found was a small abscess in the cortex of the left kidney, seen in histologic section. No acid-fast bacilli were seen.

Guinea pig: Subcutaneous or intramuscular injection of 1 mg. moist weight of bacilli into guinea pigs produced no macroscopic or microscopic lesions. No cutaneous sensitivity developed to Old Tuberculin (OT), but marked cutaneous sensitivity developed to a tuberculin prepared from the homologous acid-fast strain.

Intraperitoneal injection of 1 mg., moist weight, of bacilli suspended in saline into guinea pigs again produced no lesions after four months, although a low degree of sensitivity to OT developed. When the bacilli were suspended in 5 per cent hog gastric mucin and injected intraperitoneally, a higher degree of cutaneous sensitivity to OT was developed by guinea pigs as well as very strong sensitivity to the tuberculin from the homologous organism. Apart from some peritoneal adhesions, almost certainly the result of the gastric mucin, no lesions were found four months after injection.

Intravenous injection of 0.75 ml. of a four-day Tween®-albumin culture did not give rise to any obvious disease after sixty days or seven months. A low degree of sensitivity to OT developed when the animals were tested fifteen days after injection. At post-mortem examination, the only abnormalities found were a few microscopic abscesses in the left kidney. No acid-fast bacilli were seen, but cultures from the kidneys were" positive," even after seven months.

Orkney vole: Intraperitoneal injection of 0.1 ml. of a four-day Tween®-albumin culture into Orkney voles did not produce any obvious disease in five months. However, at post-mortem examination, one of the animals showed an abscess of the right epididymis, in which large numbers of acid-fast bacilli were present both in a direct smear and in cultures.

White mice: Mice injected either intravenously or intraperitoneally with cultures of the acid-fast microorganism all died, with the exception of some which were killed in order to obtain material for histologic study. The dose injected intraperitoneally was 0.5 ml. of a five-day culture in Tween®-albumin medium with or without the addition of hog gastric mucin. The intravenous dose varied from 0.01 to 0.2 ml. of a five-day culture in Tween®-albumin medium.

Intraperitoneal inoculation of mice: Twenty-four mice were inoculated intraperitoneally: 12 with 0.5 ml. of a Tween®-albumin culture and 12 with the same quantity of Tween®-albumin culture centrifuged at high speed and the bacilli then resuspended in 5 per cent hog gastric mucin. The animals which received the microorganisms in the culture-medium vehicle died in three to sixty-six days; those which received the bacilli in hog gastric mucin died in two to thirty-one days. This wide spread of survival time does not reveal the true picture after intraperitoneal injection of the bacilli. Whereas only 3 of the 12 mice which received the bacilli in Tween®-albumin medium were dead in ten

days, 10 of the 12 which received the bacilli in gastric mucin were dead in ten days. Six mice which received hog gastric mucin alone remained alive and well.

The post-mortem findings in the mice which died within three days of injection showed a generalized peritonitis with a clear pleural effusion. Those living more than three days showed disease of the abdominal viscera. The liver and spleen were covered with white cheesy material, although the cut surfaces of both organs looked natural. The kidneys consisted almost entirely of white cheesy material in which enormous numbers of acid-fast bacilli were present. The liver and spleen contained only a few acid-fast bacilli.

Intravenous inoculation of mice: Fifty-nine mice were inoculated intravenously with doses which varied from 0.01 ml. to 0.2 ml. of a five-day Tween®-albumin culture. Six mice received 0.01 ml. of culture. One died fifteen days after injection; the other 5 were killed twenty-nine days after injection. Before death all of the mice showed a rolling of the head from side to side and an obvious impairment of balance. All of the mice showed grossly abnormal kidneys. The other organs were macroscopically and microscopically natural.

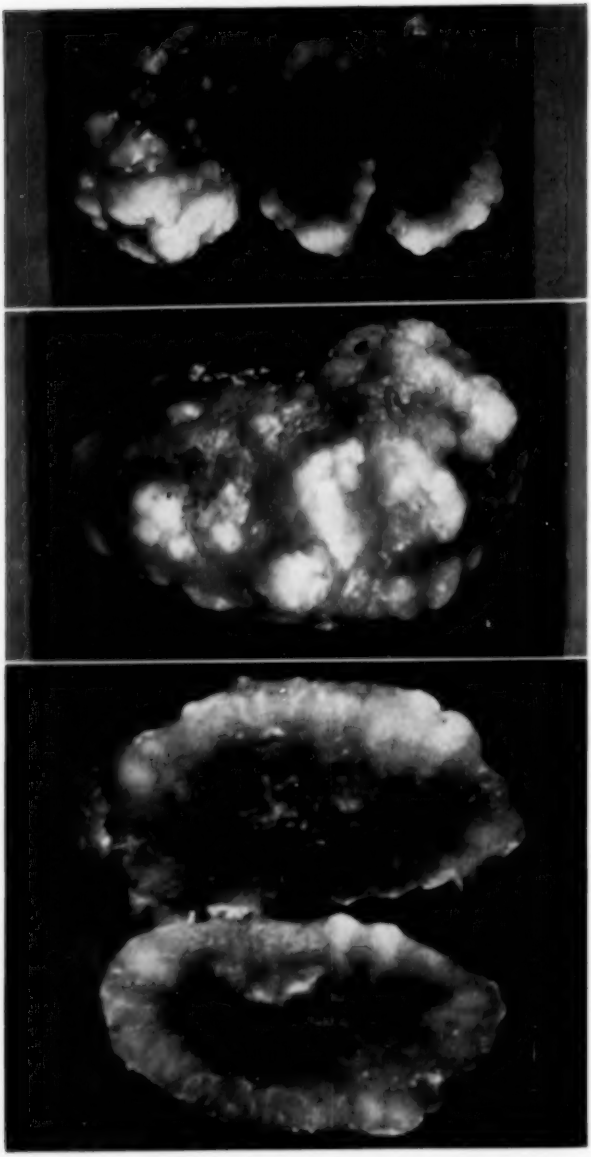
Twelve mice received 0.1 ml. of culture. Eight died in nine to seventeen days; the remaining 4 were killed twenty-two days after injection. In all 12 mice, disease was confined to the kidneys, with 2 exceptions. One of these had, in addition to the kidney disease, a clear pleural effusion; the other had a chain of cheesy lesions on either side of the abdominal aorta.

Forty-eight mice received 0.2 ml. of a five-day Tween®-albumin culture. Twenty-two were killed; the remaining 26 died in four to twenty-eight days. In all but 2 animals, the disease was confined to the kidneys. One had a clear pleural effusion, and the other had discrete pinhead lesions scattered throughout the lungs.

Macroscopically, the appearance of the kidneys was that of multiple pyemic abscesses (figures 6–8). The abscesses were scattered throughout the organ. Microscopically, the lesions did not differ from those seen in any pyemic abscess. Enormous numbers of acid-fast bacilli were present, particularly in the tubules.

Eight strains of *M. fortuitum* were sent to us by Dr. Ruth Gordon. Three of these were isolated from human abscesses: one from sputum, two from bovine mastitis, and two from soil. Intravenous injection of seven of these strains in mice gave an essentially similar result to that already described as following injection of the strain of the present study. The principal deviation from this common pattern occurred with one strain isolated from soil. This strain did not kill any of 12 mice injected, although there were small lesions in one kidney of one mouse which was killed fourteen days after injection. Only one strain isolated from sputum was as pathogenic for mice as the strain of the present study, all 6 mice which were injected being dead in ten days. Extensive disease, however, was present in the kidneys of all mice injected with six of the strains obtained from Dr. Gordon. The rolling of the head and impairment of balance already noted were also present.

No evidence of any toxic substance in the bacillus has been found. Neither a filtrate of a broth culture nor a filtrate of a heavy suspension of bacilli shaken in



FIGS. 6–8. Representative examples of kidneys from mice fourteen days after intravenous injection of 0.2 ml. of a four-day Tween®-albumin culture of *M. fortuitum*.

the cold in a Mickle shaker at 100 strokes per second for five minutes produced any toxic symptoms when injected in 0.5 ml. amounts intravenously into mice.

DISCUSSION

The classification of the mycobacteria other than *Mycobacterium tuberculosis* has for many years been unsatisfactory. It has long been recognized that mycobacteria, always excepting the classical tubercle bacilli, can occasionally produce disease in man. The morphologic and cultural characters and the pathogenicity for laboratory animals of these species vary so greatly that classification into other than wide groups is difficult. The species, *Mycobacterium fortuitum*, described by Gordon and associates (2, 3) does, however, seem to gather under one name several strains with fairly clearly defined characters. The acid-fast bacillus described in the present paper appears to fall into this group defined by Gordon and associates, although the present strain has certain characters not hitherto mentioned in the published description of other members of the species.

The type species which was described by Cruz (4) was isolated from abscesses in the arm of a woman following the injection of a preparation containing vitamins A and D. He describes the bacillus as easily cultured in the usual media, colonies appearing in three days on agar. It was not pathogenic to guinea pigs, cats, or hamsters even when large quantities were injected subcutaneously, intravenously, or intraperitoneally. It was not pathogenic to the rabbit when injected intravenously or intraperitoneally, but gave rise to a local abscess five to eight days after subcutaneous inoculation. The abscess wall, both in man and rabbits, showed histologic lesions resembling those of pseudotuberculosis.

Penso and co-workers (5) described a new species, which they called *Mycobacterium minetti*. The strains which they describe had been isolated by Minett (6) from cattle which had reacted to avian tuberculin and occasionally to bovine tuberculin. The species is described in great detail. Their description of *M. minetti*, except in a few details, agrees very closely with the strain described in the present paper. It seems that it should be grouped with *M. fortuitum*. The morphologic, cultural, and pathogenic properties of *M. minetti* are seen from their description and beautiful illustrations to be almost identical with those of *M. fortuitum*. The differences between the two strains are (*1*) a slightly greater heat resistance of *M. minetti*, (*2*) a higher resistance to antimicrobial drugs of *M. minetti*, and (*3*) a greater pathogenicity of *M. minetti* for guinea pigs and rabbits. Penso and his colleagues (5) show that the rolling of the head and impairment of balance seen in infected mice, and also seen by them in rabbits, is due to an infection of the middle ear.

The localization of *M. fortuitum* in the kidneys of mice after intravenous injection is an unusual and interesting feature. It appears from experiments in progress that the bacillus is unable to multiply in the organs of the mouse, with the exception of the kidneys. This organ specificity has been noticed with other bacteria. Gorrill (7) showed that the intravenous injection of *Ps. pyocyanea* into mice results either in septicemic death in twenty-four to forty-eight hours or in the formation of renal abscesses which killed the mice in three to fourteen

days, or in survival. The results depend on the number of bacteria injected and the strain of *Ps. pyocyanea*. A dose varying from 20 to 100 millions of bacteria of twelve strains showed death of the injected mice in three to fourteen days with renal abscesses. Approximately double the dose of each strain resulted in a septicemic death in twenty-four to forty-eight hours without focal lesions in the kidneys. The writers have not seen septicemic death following the injection of *M. fortuitum*, but possibly this might occur if an enormous dose of bacilli were injected intravenously.

Gorrill also noted what he calls "rolling disease" in some of the mice injected with *Ps. pyocyanea*. The condition he describes is very similar to that seen in mice after injection of *M. fortuitum*. He has also reported (8) multiple abscesses in the kidneys of mice after the intravenous injection of coagulose plus staphylococci.

SUMMARY

An acid-fast bacillus isolated from the cervical lymph nodes of a young Maltese woman is described.

The bacillus has the cultural characteristics of *Mycobacterium fortuitum*. It is pathogenic for mice. Intravenous injection in mice resulted in multiple abscesses in the kidneys, the other organs being free of disease.

SUMARIO

Mycobacterium Fortuitum

Describese un bacilo ácidorresistente aislado de los ganglios linfáticos cervicales de una joven de Malta.

El bacilo muestra las caracteristicas culturales del *Mycobacterium fortuitum*. Patógeno para los ratones, la inyección intravenosa en éstos da por resultado abscesos múltiples en los riñones, permaneciendo indemnes los demás órganos.

RESUME

Mycobacterium Fortuitum

Description d'un bacille acido-résistant isolé à partir d'un ganglion cervical chez une jeune Maltaise.

Le bacille possède les caractères culturaux du *Mycobacterium fortuitum*. Il est pathogène pour la souris. L'injection intraveineuse chez la souris détermine de multiples abcès dans les reins. Les autres organes demeurent indemnes de l'affection.

Acknowledgment

The writers wish to express their thanks to Dr. A. H. T. Robb-Smith for his report on the histologic examination of the lymph gland; to Dr. J. Galea, Chief Government Medical Officer, Malta; to Professor A. J. Craig, Senior Surgeon, St. Luke's Hospital, Malta; and to Professor G. Xuereb, Pathological Department, St. Luke's Hospital, Malta.

The writers are indebted to Dr. Ruth Gordon, not only for confirming the cultural characters, but also for her courtesy in sending us several strains of *M. fortuitum* and for allowing us to read the paper by herself and her colleagues on *M. fortuitum* before publication (3).

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NYSTATIN (MYCOSTATIN®) THERAPY IN EXPERIMENTAL COCCIDIOIDOMYCOSIS[1,2,3]

LEE E. GORDON, CHARLES E. SMITH, AND DONALD S. WEDIN

(Received for publication February 28, 1955)

INTRODUCTION

The treatment of coccidioidomycosis with the antimicrobial drugs which are presently available has not been successful. Therapeutic trials with other drugs have likewise been ineffective or inconclusive. Among the drugs currently used, ethyl vanillate (1–3) is frequently employed, but the necessity for the daily administration of 90 or 100 half-gram capsules greatly limits its applicability, especially in patients with meningitis.

Hazen and Brown (4) reported that an antimicrobial substance produced by a soil actinomycete, first named "fungicidin" and now called nystatin (Mycostatin®), inhibited the growth of *Coccidioides immitis* at a concentration of 6.25 γ per ml. in glucose tryptone broth. Cohen and Webb (5) reported that nystatin incorporated in Sabouraud's medium at a concentration of 7.5 γ per ml. completely inhibited the mycelial growth of *C. immitis*. Newcomer and associates (6) confirmed the *in vitro* inhibition of *C. immitis* at 1.56 to 6.25 γ per ml., and reported that the drug favorably influenced the course of the disease in mice injected intraperitoneally with spherules of the fungus. Campbell and co-workers (7) likewise reported an *in vivo* activity of nystatin in the form of a marked reduction in mortality in mice with experimental histoplasmosis when early and adequate treatment with nystatin was given.

The present report is a further exploration of the efficacy of nystatin in the treatment of experimentally induced coccidioidal infections in mice.

MATERIALS AND METHODS

In vitro test: Ten milligrams of nystatin[4] were added to 10.0 ml. of sterile asparagine medium (8). Duplicate serial dilutions were then made with asparagine medium. One drop of a saline spore suspension of *Coccidioides immitis* strain Silveira which contained approximately twenty viable spores was added to each tube. These were incubated at 37°C. and observed for growth during a thirty-day period. Two lots of nystatin were used. Both were tested for *in vitro* fungistatic activity.

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[1] From the School of Public Health and Naval Biological Laboratory, Department of Bacteriology, University of California, Berkeley 4, California.

[2] These investigations were conducted in part under the sponsorship of The Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board; and were supported, in part, by the Office of the Surgeon General, Department of the Army and, in part, by a contract between the University of California and the Office of Naval Research.

[3] The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1252, U. S. Navy Regulations, 1948).

[4] Generously supplied as Mycostatin® by Dr. Richard Donovick, Squibb Institute for Medical Research, New Brunswick, New Jersey.

In vivo test: Infection technique. White male mice, Namru strain (9), weighing approximately 25 gm., were inoculated intraperitoneally with 0.5 ml. of a spore suspension of *Coccidioides immitis* strain Silveira. Plate counts of the inoculum used in each experiment were made and found to be 170 to 750 spores per 0.5 ml.

Nystatin: The nystatin was prepared by adding a weighed amount of the crystalline preparation to sterile buffered saline (pH 7.4) so that 0.5 ml. contained 0.5 mg., 1.0 mg., and 2.0 mg. The drug was administered by subcutaneous injection. It was found that healthy 25-gm. mice tolerated daily injections of 2.0 mg. solution of nystatin for ten days.

Treatment with nystatin at time of infection: Two infected groups of 100 mice each were treated with nystatin beginning on the day of infection. One group received 0.5 mg. and the other 1.0 mg. daily for five consecutive days. Then the animals were allowed to rest for two days. This schedule of treatment was followed for thirty days. Similarly, 50 infected animals received daily injections of 0.5 ml. of buffered physiologic saline solution. The experiment was terminated forty-five days after therapy was discontinued or seventy-five days after infection. Autopsies were performed on animals which died and on those which survived to the end of the experiment, when they were sacrificed. The lungs, liver, spleen, and mesentery were examined for gross evidence of infection.

Delayed nystatin treatment: Other infected animals were divided into seven groups of 25 each. At intervals of two, four, six, eight, ten, and twelve days after infection, nystatin treatment was instituted. All mice received 1.0 mg. daily for five days, followed by a rest period of two days. This schedule was continued for thirty days. A control group of 25 infected mice similarly were given 0.5 ml. sterile buffered physiologic saline solution. The survivors in each group were sacrificed and autopsied seventy days after infection.

Two additional groups of infected animals, each containing 100 mice, were observed for the earliest signs of severe coccidioidomycosis. The onset of deaths with extensive lesions in the lungs and viscera was the criterion used for commencing therapy. On the fourteenth day after infection, 8 of the mice, which had been designated as controls to be injected with the physiologic saline solution, and 7 of the mice to be treated with 2.0 mg. of nystatin daily died, so treatment was begun. Treatment was given for five consecutive days, followed by two days of rest. After the fourteenth day of therapy the drug dosage was reduced to one-half the amount, and treatment was continued for an additional sixteen days. The experiment was terminated thirty days after the discontinuation of therapy or seventy-four days after infection. The mice were autopsied as previously described.

RESULTS

Nystatin was inhibitory *in vitro* for *Coccidioides immitis* in concentrations of 100 γ per ml. It may be seen in table 1 that growth was suppressed in a concentration of 10 γ per ml. during the early days of incubation, but, in ten days, growth occurred.

A second lot of drug which was used was tested and found to have the same spectrum of *in vitro* inhibition as the first.

The results of the studies *in vivo* are plotted as daily cumulative percentage mortality in a manner described by Friedman, Smith, and Gordon (10) for the assay of virulence of *C. immitis*. As shown in figure 1, the mortality of mice in which treatment was begun at time of infection, maintained for thirty days, and observed for an additional forty-five days, was 13 per cent among the 100 animals treated daily with 1.0 mg. of nystatin and 28 per cent among the 100 treated daily with 0.5 mg. All 50 control animals died within the experimental period.

The slopes of the mortality curves (figure 1) gave further evidence of the bene-

TABLE 1

INHIBITION OF GROWTH OF COCCIDIOIDES IMMITIS IN ASPARAGINE MEDIUM CONTAINING NYSTATIN

Concentration of Drug per cc. Ml.	Extent of Growth*									
	Days									
	5		10		15		20		30	
0										
1,000.0	–	–	–	–	–	–	–	–	–	–
100.0	–	–	–	–	–	–	–	–	+++	–
10.0	–	–	++	++	++++	++++	++++	++++	++++	++++
1.0	++	+++	++++	+++	++++	++++	++++	++++	++++	++++
0.1	++	++++	++++	++++	++++	++++	++++	++++	++++	++++

* Legend
– = no growth
++–++++ = moderate to heavy growth

ficial effect of the nystatin. All of the treated and untreated animals which died had extensive pulmonary and visceral lesions. Lesions with viable *C. immitis* were present in animals sacrificed at the end of the experiment. Their pulmonary lesions were fewer than in the mice which had died, but the visceral lesions seemed as extensive.

In figure 2 the mortality is compared when treatment using 1.0 mg. of nystatin was delayed. No deaths occurred among the 25 mice in which treatment was delayed for a two-day period after infection. Only one death (4 per cent) occurred among the 25 animals in which treatment was delayed for a period of four days following infection. Death occurred in 4 (16 per cent) of the 25 mice in which treatment was delayed six days and in 8 (32 per cent) of the 25 animals in which nystatin injections were begun eight days after infection. The mortality of the mice in which treatment was delayed ten and twelve days after infection was the same, 16 (75 per cent) among each group of 25. For the control group of 50 mice, 46 (92 per cent) died spontaneously in the seventy-five-day period of total observation after infection.

It may be noted that the few deaths in the six- and eight-day delayed treatment experiments occurred for the most part after the treatments were stopped. Moreover, the deaths in the ten- and twelve-day delayed treatment studies diminished during the latter part of the treatment but resumed after treatment was stopped and continued during the period of observation.

In the very rigorous test of withholding the drug for fourteen days, at a time when the mice were beginning to die, the dosage of 2.0 mg. soon was associated with a reduction in mortality. However, the mice continued to die during the entire period of treatment and subsequent observation. Fifty per cent of the 100 control animals were dead by the eighteenth day, whereas twenty-nine days elapsed after infection before 50 per cent of the treated mice died. Three-quarters of the control mice were dead in twenty-three days; the same proportion of the treated mice required fifty-one days to accumulate a similar number of

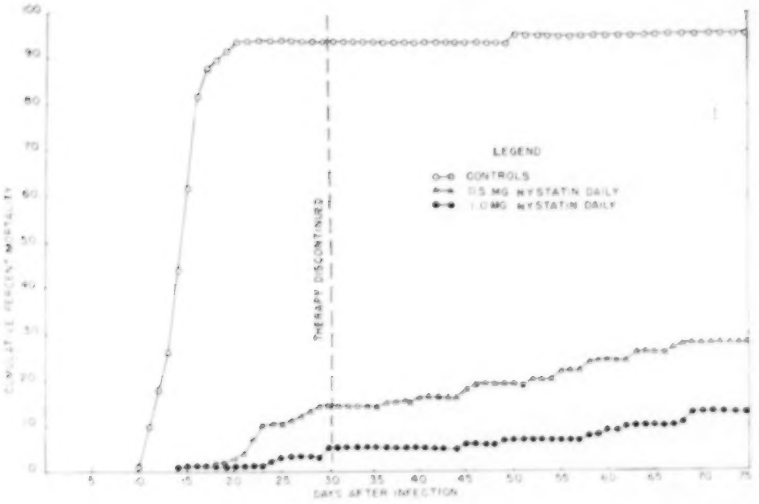

FIG. 1. The treatment of coccidioidomycosis in mice with nystatin given immediately after infection.

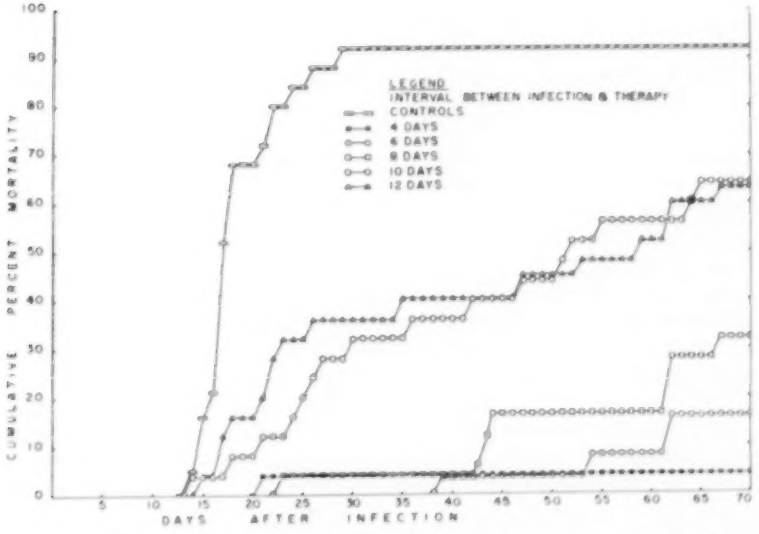

FIG. 2. The effect of delayed nystatin therapy on coccidioidomycosis in mice.

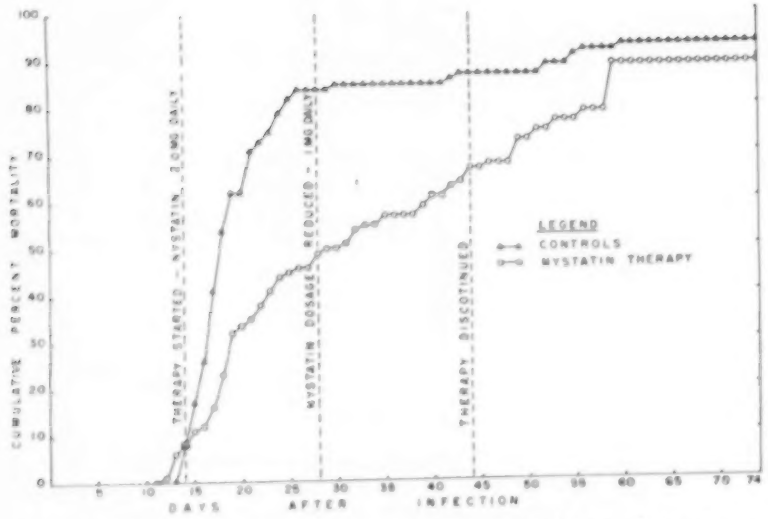

FIG. 3. The effect of nystatin therapy on severe coccidioidomycosis in mice.

deaths. However, by the end of the seventy-four days, 93 per cent of the control animals and 89 per cent of the treated mice had died.

DISCUSSION

These experimental results are encouraging but, like so many other drugs tried against disseminated coccidioidomycosis, nystatin does not yet seem to represent an unqualifiedly successful chemotherapy.

The studies *in vitro* in the present investigation indicate that a higher concentration of nystatin is necessary for continued fungistasis than was observed by Hazen and Brown (4), Cohen and Webb (5), and Newcomer and associates (6). Perhaps the continued period of observation in the present study explains the need for higher concentration than the 1.56 to 7.5 γ inhibitory concentration previously reported.

When mice were treated with nystatin immediately upon infection with *Coccidioides immitis*, the results were very encouraging. Even when the drug was withheld for a four-day period following infection, the chemotherapy appeared to be effective. When the microorganisms were well disseminated throughout the mice, and their condition ten to twelve days after infection was comparable to that of the seriously ill patient with disseminated disease, there still appeared to be some benefit from nystatin therapy; but the benefit was limited. The results when treatment was delayed fourteen days and the mice were actually dying revealed an even poorer capacity to control the infection.

It is unrealistic to hope that antimicrobial therapy might be administered from the very beginning of a human coccidioidal infection. Fortunately, however,

human coccidioidomycosis is characteristically benign (11). Indeed, with such variety in clinical manifestations and course of the primary infection, evaluation of a therapeutic agent in primary disease is very difficult. Possibly nystatin might be tried in cases of human disease with an ominously high titer of complement fixation (12). Even in such patients, however, the course of the disease is so variable that interpretation would have to be cautious.

The present studies support those of Newcomer and associates (6) in warranting the clinical trials now under way. The suspicion remains, however, that, as with ethyl vanillate, it will be found that the usefulness of nystatin is limited. Accordingly, the search for an effective antimicrobial drug against *Coccidioides immitis* should be continued.

SUMMARY

Nystatin (Mycostatin®) was shown to be inhibitory for *Coccidioides immitis* in *in vitro* concentrations of 100 γ per ml. of asparagine medium.

Mice infected intraperitoneally with a saline spore suspension of *C. immitis* and treated soon after infection with repeated dosages of 1.0 mg. of nystatin showed a marked reduction in mortality. When treatment was delayed beyond the eighth day after infection, the results were less favorable. All treated and untreated animals which died had extensive pulmonary and visceral lesions. Treated animals sacrificed at the end of the experiment had fewer pulmonary lesions, but the visceral lesions were extensive.

Although these experimental results are encouraging, the search for additional antimicrobial drugs for coccidioidomycosis should be continued.

SUMARIO

La Terapéutica con Nistatina (Micostatina®) en la Coccidioidomicosis Experimental

La nistatina (Micostatina®) se mostró inhibidora *in vitro* para el *Coccidioides immitis* a concentraciones de 100 γ por ml. de medio de asparagina.

Los ratones infectados intraperitonealmente con una suspensión salina de esporos de *C. immitis* y tratados poco después de la infección con dosis repetidas de 1.0 mg. de nistatina revelaron una notable reducción de la mortalidad. Cuando se demoró el tratamiento hasta más allá del octavo día consecutivo a la infección, los resultados fueron menos favorables. Todos los animales, ya tratados o no, que murieron tenían extensas lesiones pulmonares y viscerales. Los animales tratados sacrificados al terminar el experimento tenían menos lesiones pulmonares, pero las viscerales eran extensas.

Aunque estos resultados experimentales son alentadores, debe continuar la busca de otros agentes antimicrobianos y quimioterapéuticos contra la coccidioidomicosis.

RESUME

Nystatine (Mycostatin®) dans le traitement de la coccidioidomycose expérimentale

La nystatine (mycostatin®) à la concentration de 100 γ par ml. s'est montrée inhibitrice pour le *Coccidioides immitis in vitro* dans un milieu contenant de l'asparagine. Des souris, soumises à l'inoculation intrapéritonéale d'une suspension de spores de *C. immitis* dans le sérum physiologique après avoir été traitées aussitôt après l'infection avec des doses répétées de 1 mg. de nystatine, ont présenté une réduction marquée de la mortalité. Quand le traitement était retardé au-delà du huitième jour après l'infection, les résultats

étaient moins favorables. Tous les animaux ayant succombé, traités et non traités, étaient porteurs de lésions viscérales et pulmonaires extensives. Chez l'animal traité et sacrifié à la fin de l'expérimentation, les lésions pulmonaires étaient moins nombreuses, mais les lésions viscérales étaient extensives.

Bien que ces résultats expérimentaux soient encourageants, il y a lieu de poursuivre la recherche d'autres agents antimicrobiens et chimiothérapeutiques contre la coccidioidomycose.

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HEPATIC DAMAGE IN CHRONIC PULMONARY TUBERCULOSIS[1,2]

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INTRODUCTION

Hepatic damage is a frequent finding at autopsy in tuberculosis, while clinical manifestation of liver involvement in pulmonary tuberculosis is rare. Lichtman (1) rightly points out that, in chronic pulmonary tuberculosis, clinical observations demonstrate a lack of parenchymal hepatic damage, contrary to the presence of such hepatic damage as revealed by sensitive hepatic tests and further corroborated by autopsy findings.

Review of the available literature on this subject of structural and functional hepatic changes reveals a paucity of material. Furthermore, opinions are divided and the tests used in the past are not readily comparable with the present recognized techniques.

The functional hepatic damage in chronic pulmonary tuberculosis was studied by Leurete and associates (2*a*), Steidl and Heise (3), O'Connor and associates (4), Kerger and Garber (5), Hurst, Maier, and Lough (6), Seibert and Nelson (7), Galen, Weiner, and Hartman (8), Savacool (9), and Reddy and associates (25). From a perusal of the above literature, it seems that some evidence of functional hepatic damage was detected in a substantial proportion of tuberculous patients. Disturbance of albumin and globulin ratio, abnormality in cephalin cholesterol, and bromsulfalein retention tests have been outstanding in the various studies.

The morbid anatomy of the liver in tuberculosis has been studied in postmortem examinations by Simmon (2*b*), Levenson and Karsner (10), Leurete (2*a*), Begtrup (24), Saphir (11), and Florentin and associates (12). Tubercles in the liver were found in 80 to 100 per cent of the patients. Fatty infiltrations were found in various stages in 25 per cent of the patients. An increase in periportal connective tissue, which has been labeled hepatic sclerosis, has also been mentioned.

To the best of the knowledge of the present writer, no attempt has yet been made to correlate the functional hepatic damage, as demonstrated by liver function tests, with the structural changes which occur in the liver in patients with pulmonary tuberculosis. With the advancement in the field of therapy of tuberculosis with the various antimicrobial agents like streptomycin, para-aminosalicylic acid (PAS), and isoniazid, the question of a long-range effect of these drugs, with particular reference to the functional capacity of the liver, has likewise not been studied. By the same token, it is known that changes in the liver may be induced experimentally in animals by anoxemia, malnutrition, and various hepatotoxic infective or chemical agents.

[1] From the Delhi Tuberculosis Clinic, Queens Road, Delhi 6, India.

[2] Portions of the present work have been submitted as a thesis for Doctor of Medicine in the University of Agra.

Accordingly, it was planned to study the problem of hepatic damage in pulmonary tuberculosis and in other chronic nontuberculous pulmonary conditions. The latter conditions have been included to serve as a basis for comparison in determining whether such damage is due to: chronic, nonspecific inflammatory lesions; malnutrition; or a pure and simple result of tuberculous toxemia or bacteremia.

PLAN OF INVESTIGATION

It was decided to carry out the investigation along lines recommended by the Indian Medical Research Council. The protocols were planned on the basis of the case sheets recommended by the Council, but due consideration was given to the information demanded by the problem in hand.

The material was collected from the various wards of the Jaya Arogya Hospital attached to the Gajra Raja Medical College. Patients who had already suffered from liver disease, jaundice, occupational exposures, and acknowledged hepatotoxic drugs, including alcohol, were excluded from the present study.

Case Material

Group I (nontuberculous pulmonary disease): Group I consisted of 34 patients who were suffering from various chronic nontuberculous pulmonary diseases. These patients also served in a sense as controls. The collection of fasting blood for hepatic function tests and the liver biopsy were always carried out on the same day. Particular care was taken to classify the case as nontuberculous by the following criteria:

Thorough clinical examination
Sputum negative for acid-fast bacilli on microscopy of two concentrated specimens
Roentgenographic examination
Correlation of the progress of the patient with the above data during his stay in the hospital

Group II (pulmonary tuberculosis): Group II included 35 patients with pulmonary tuberculosis who had received no treatment. The collection of the fasting blood and the biopsy were performed on the same day, as in the studies with *Group I*.

In addition, *Group II* included 25 patients with pulmonary tuberculosis who had been treated in the tuberculosis wards for various periods. Effort was made to collect a sufficient number of patients who had been treated with streptomycin-PAS.

All patients were hospitalized; their ages varied from eighteen to sixty-five years. Males and females were almost equally distributed in the group with pulmonary tuberculosis, while there were approximately twice as many males in the nontuberculous group.

Of 60 patients with pulmonary tuberculosis, 46 had far advanced and 14 had moderately advanced disease. None had minimal disease.

MATERIALS AND METHODS

Various tests have been proposed to detect the hepatic damage. Franklin and associates (13) recommended the following scheme and suggested that at least one test from each group should be carried out.

For detection of decreased metabolic activity:
(*a*) Total serum cholesterol and cholesterol ester formation
(*b*) Serum albumin
(*c*) Bromsulfalein retention test
For detection of a disturbance of bile excretion:
(*a*) Total serum bilirubin
(*b*) Serum alkaline phosphatase
For detection of necrotizing and inflammatory changes:
(*a*) Cephalin cholesterol
(*b*) Thymol turbidity
(*c*) Determination of serum globulin

As the central purpose of the study was to measure all three phases, i.e., metabolic activity, bile excretion, and necrotizing and inflammatory changes, all of the above tests were carried out.

OBSERVATIONS

Tests of Hepatic Function

Bromsulfalein retention test

A 5 mg. per kg. body weight dose of bromsulfalein has been used. Five milliliters of the fasting blood were collected after the syringe had been washed with normal saline (to prevent hemolysis), and the calculated amount of the dye was injected through the same needle in the form of a 5 per cent solution. After forty-five minutes, a specimen of blood was collected from the opposite antecubital vein. Detection of the dye was carried out by a photoelectric colorimeter, using a yellow filter, and the results were calculated in terms of percentage retention of the dye.

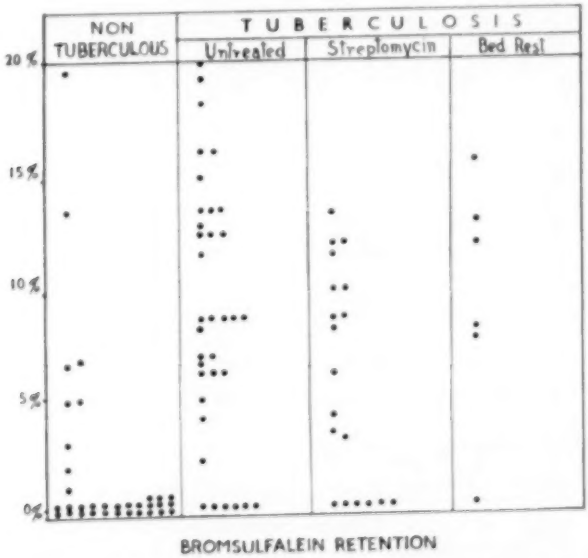

FIG. 1. Graph showing the results of bromsulfalein retention test.

The results with the bromsulfalein tests (figure 1) should be interpreted with considerable reservations, for, of the 60 patients in *Group II* (pulmonary tuberculosis), only 12 were afebrile. The remainder were having daily temperatures which ranged from 99.5° to 103.5° F. in some cases. In the nontuberculous series (*Group I*), 18 of the 34 patients were febrile. With due recognition of the important influence of a febrile illness on the bromsulfalein retention, it can be stated that 85 per cent of the tuberculous patients showed more than 5 per cent bromsulfalein retention. Abnormal values were present in 5 of the 34 nontuberculous patients, in 25 of the 35 untreated tuberculous patients, and in 15 of the 25 treated tuberculous patients.

Thymol turbidity

Neefe (14) is of the opinion that values above 2 units for the thymol turbidity test are abnormal. McLaglen (18c) also agrees that, in normal healthy subjects, values higher than 2 units are not encountered. Nevertheless, in the present writer's experience with hospitalized patients who are not suffering from hepatic disease, the range of thymol turbidity is quite frequently between 2 and 4 units.

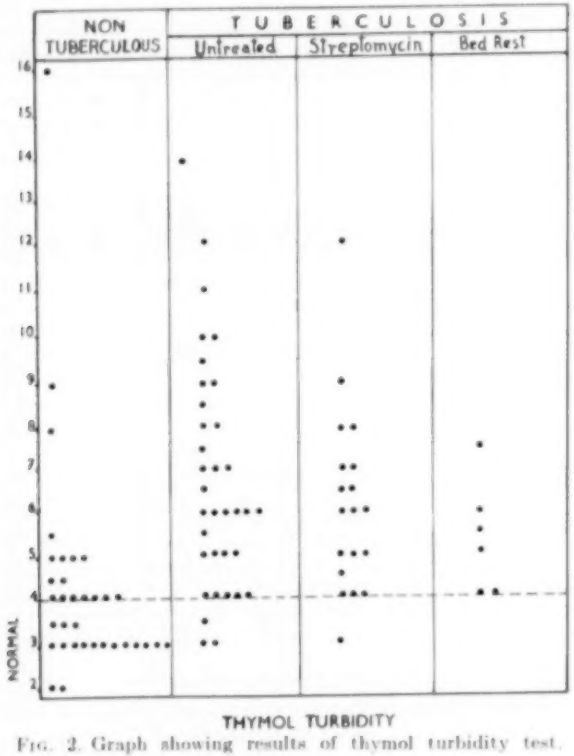

FIG. 2. Graph showing results of thymol turbidity test.

The same technique for determination of thymol turbidity was followed which is described on page 91 of *Microchemical Analysis in Medical Biochemistry* by E. J. King (15). The results are tabulated in figure 2.

A value for thymol turbidity of greater than 4 units was present in 10 of the 34 nontuberculous patients and in 45 of the 60 patients with pulmonary tuberculosis. Such abnormal values were found in 28 of the 35 untreated patients and in 17 of the 25 treated patients.

Cephalin cholesterol

The antigen for the cephalin cholesterol test was obtained from the Difco Laboratories. It was dissolved in 5 ml. of ether, and 1 ml. of the above stock solution was added to 35 ml. of distilled water. The solution was slowly heated and permitted to simmer until the final solution was exactly 30 ml. The emulsion so prepared was kept in the refrigerator, and the test tubes were kept in a dark place. No exposure to air and light was allowed.

The results of the cephalin cholesterol determinations are graded from 1 to 4 units. Complete precipitation with a clear supernatant fluid was designated as 4 units. Whenever a fresh emulsion was prepared, it was compared with a negative as well as a positive control. Moreover, the stock emulsion was checked once in a fortnight against negative and positive controls.

The value for the cephalin cholesterol in healthy normal subjects ranges around 1 unit.

The results of the cephalin cholesterol determinations are presented in figure 3.

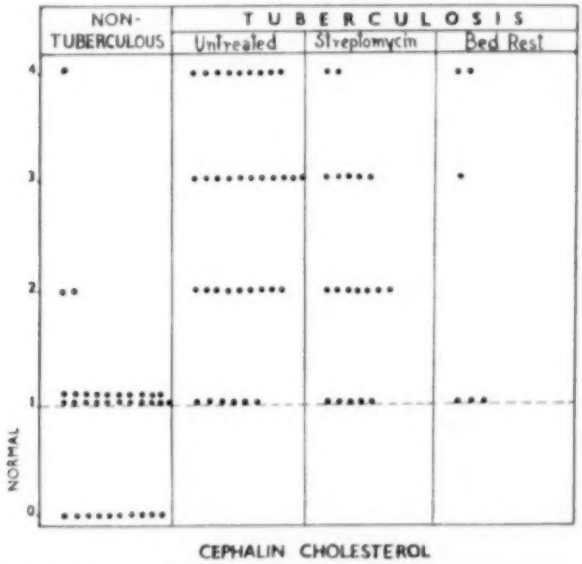

FIG. 3. Graph showing results of cephalin cholesterol test.

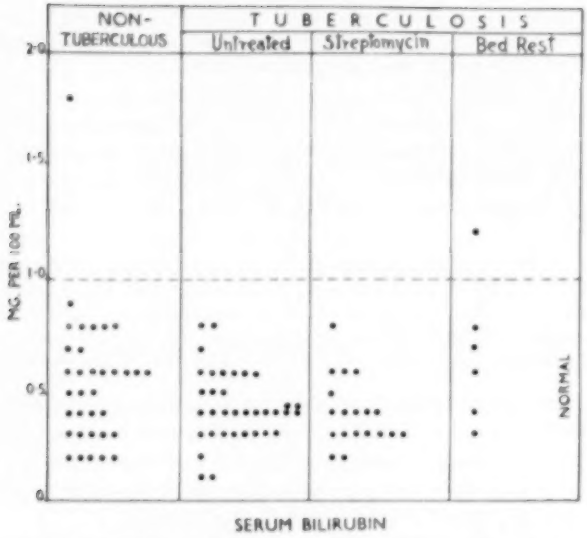

FIG. 4. Graph showing the results of serum bilirubin test.

Values of more than 1-plus flocculation were present in 3 of the 34 nontuberculous patients. In the tuberculous group, abnormal values were found in 18 of the 25 patients who were receiving antimicrobial therapy and in 29 of the 35 patients who had received no chemotherapy.

Serum bilirubin

Values for serum bilirubin between 1 and 2 mg. per 100 ml. were found in one nontuberculous and in one tuberculous patient. The latter patient was receiving chemotherapy. The values for all of the patients are presented in figure 4. The technique used was the one described by King (15, page 3).

Alkaline phosphatase

The results of the alkaline phosphatase determination are expressed as King-Armstrong units, which is defined as the amount of the enzyme which will set free 1 mg. of the phenol in a given time under conditions of the test. Readings above 10 units are taken as an indication of hepatic dysfunction. The technique used is the same as the one described by King (15, page 70).

The results are presented in figure 5. As may be seen in the figure, there were no instances in which abnormal values were found for the alkaline phosphatase in either the tuberculous or the nontuberculous patients.

Protein fractionation

The fractionation was carried out by the process of nesslerization, as described by King (15, page 41). The mean average of the serum protein values was 5.85

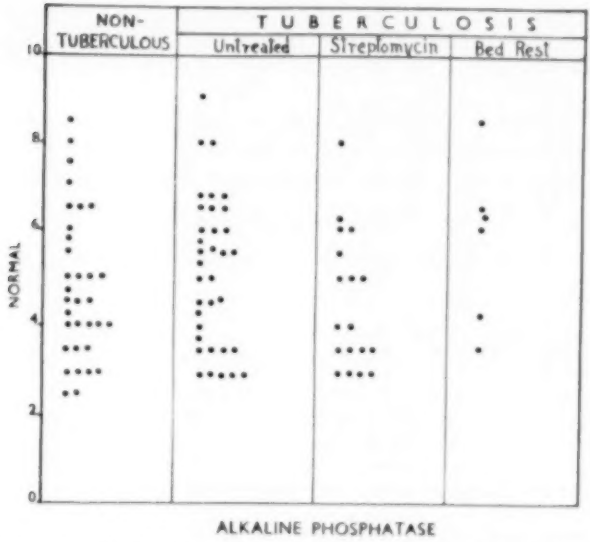

FIG. 5. Graph showing serum alkaline phosphatase determination.

TABLE 1

MEAN AVERAGES OF PROTEIN FRACTIONATION

	Total Protein		Total Albumin		Total Globulin	
	Mean Average	Standard Deviation	Mean Average	Standard Deviation	Mean Average	Standard Deviation
Pleurisy with effusion	5.46	0.300	3.12	0.330	2.34	0.672
Lung abscess	5.91	1.130	3.56	0.770	2.36	0.436
Bronchiectasis	6.06	0.500	3.75	0.594	2.33	0.230
Pulmonary tuberculosis, previously untreated (normal)	5.21	0.827	2.85	0.590	2.45	0.470
Pulmonary tuberculosis, previously untreated (abnormal)	5.17	0.703	2.88	0.473	2.50	0.453
Pulmonary tuberculosis, streptomycin (abnormal)	5.62	0.815	2.87	0.590	2.83	0.390
Pulmonary tuberculosis, streptomycin (abnormal)	6.04	0.693	2.99	0.390	3.23	0.500
Pulmonary tuberculosis, treated with bed rest only	5.25	0.800	2.70	0.850	2.55	0.460

gm. per 100 ml. in the nontuberculous patients, whereas it was 5.18 gm. per 100 ml. in the untreated patients (table 1). In the drug-treated tuberculous group, the mean average was 5.8 gm. per 100 ml. in treated tuberculous patients. Hurst, Maier, and Lough (6) and Seibert and Nelson (7) found the plasma proteins within the normal limits in pulmonary tuberculosis.

In the nontuberculous patients, the mean average of the serum albumin values was 3.45 gm. per 100 ml. In the group of tuberculous patients, the mean average value was 2.9 gm. per 100 ml. and was the same in both the treated and untreated patients.

The serum globulin mean average value was found to be 2.35 gm. per 100 ml. in the nontuberculous group, 2.5 gm. per 100 ml. in the untreated tuberculous patients, and 2.95 gm. per 100 ml. in the group of drug-treated patients.

Histopathologic Changes

Liver biopsy: Vim Silvermans' needle was used throughout the study.

Failure rate:

Total number of biopsies	94
No representative tissue	2
Small biopsy material	3
Failure rate	5.5 per cent

It was decided to attribute any mortality to the operative procedure if the patient should die within twenty-four hours of the liver biopsy. Of the total of 94 patients, one died within twenty-four hours following the biopsy. On post-mortem examination, there was no evidence of laceration of the gallbladder or of any other organ. Neither was there any evidence of internal hemorrhage. The pathologist's opinion was that biopsy was not responsible for the death.

To what extent examination of biopsy material can be relied upon as representative of structural changes present in the liver is a debatable question. Waldstein and Szant (16) carried out a very interesting study. They obtained ten specimens from different parts of each liver from fifteen post-mortem specimens and compared the histologic findings. They came to the conclusion that injury to the liver cells and fatty changes were almost always diffuse, but that alteration of lobular pattern, scarring, and inflammatory lesions showed dissimilarity in approximately 10 per cent of the specimens.

It is reasonable to suppose that liver biopsy study in which a random sample is obtained will be of value in diffuse hepatic lesions. The histologic examination should therefore always be done with reservations.

Analysis of structural changes are presented in table 2.

Fatty infiltration: Fatty infiltration of the liver was present in 2 nontuberculous patients and the process was irregular and peripheral in distribution (figures 6 and 7). In the 34 patients with untreated pulmonary tuberculosis, fatty infiltration was present in 12. In 3 of the patients, the infiltration was complete; it was periportal in 7 patients, and in 2 patients it was neither marked nor zonal. In the 19 streptomycin-treated patients, fatty infiltration of the liver was present in 8. The process was complete in one, central in one, periportal in 3, and irregular, scattered, and patchy in 3 patients.

Necrosis and regeneration of the liver cells: In the nontuberculous series, necrosis with regeneration was found in 2 cases. One of these patients died within a week, and post-mortem examination revealed a nonspecific pulmonary cyst and, in addition, acute infective necrosis (figure 7). If this case is excluded from the series, only 5 patients showed a morbid histology.

Necrosis with regeneration of the liver cells was present in 4 patients in the untreated tuberculous series; none of these showed proliferation of the bile canaliculi or the Kupffer's cells (figures 8 and 9). In the treated group, 5 patients

TABLE 2

STRUCTURAL CHANGES IN LIVER

	Nontuberculous Pulmonary Infections	Pulmonary Tuberculosis		
		Untreated	Treated	
			Streptomycin	Bed Rest
Number of cases investigated	33	34	19	6
Total number showing morbid histology	6	26	13	6
Fatty infiltration	2	12	8	2
Amyloidosis	—	1	—	—
Necrosis	2	4	1	1
Regeneration	—	2	1	—
Inflammatory cell infiltration	2	9	5	3
Focal collection of inflammatory cells	2	2	1	—
Tubercle-like structures	—	3	3	1
Tubercles	—	3	1	1
Periportal connective tissue	2	13	5	2
Condensation of connective tissue	—	6	3	1
Reticulum scaffolding	—	2	2	1
Hyperemia	2	5	3	3
Increase in cellularity of periportal connective tissue	—	2	1	—
Kupffer's cells of proliferation	—	2	—	—
Bile canaliculi proliferation	—	3	2	1

showed necrosis of the liver cells, and in 3 there was some evidence of active regeneration.

No other worker has reported necrosis of the liver cells in tuberculosis, but Saphir (11) has reported a few instances of regeneration of the liver cells and proliferation of the bile canaliculi.

Hyperemia: Hyperemia was present in the tissues from 2 patients in the nontuberculous series and in 5 patients in the untreated tuberculous series. Hyperemia was also present in the tissues of 5 patients from the treated group.

Increase in the quantity and density of the periportal connective tissues: An increase in the periportal connective tissue was present in only 2 nontuberculous patients, one of whom was suffering from acute infective necrosis as described earlier. Thirteen patients in the untreated tuberculous group showed an increase in the connective tissue, 6 of whom also showed, in addition, some increased density of the periportal connective tissue (figure 10). These findings were likewise present in 7 of the patients in the treated tuberculous group. The tissue examination in one of these patients left the impression that he had early cirrhosis of the liver of "diffuse hepatic fibrosis" type.

Levenson and Karsner (10), in a study of fifty patients suffering from pulmonary tuberculosis, could find cirrhosis in only two cases. Spring (17) and Saphir (11) do not believe that classical Laennec's cirrhosis can result from pulmonary tuberculosis. They label this increase in periportal connective tissue hepatic sclerosis. If the necrosis and active regeneration of the liver cells are

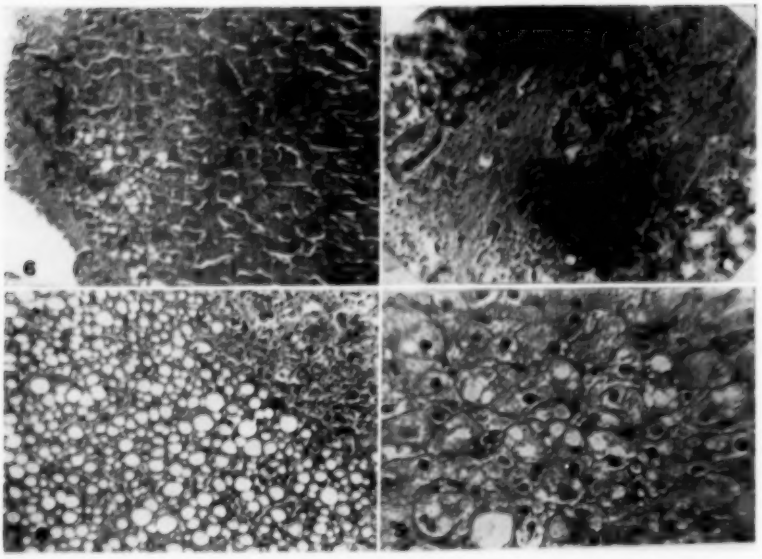

FIG. 6. Section of liver from a case of lung abscess showing patchy infiltration at the periphery of the lobule. *Case No. 25*—S-52-262. (H. & E. stain. ×175).

FIG. 7. Section of liver from a case of multiple cysts in the lung showing massive necrosis of the liver associated with early post-necrotic scarring. There are wide bands of vascular connective tissue encircling the surviving hepatic lobules. Cells at the periphery of the pseudo-lobule show a minor degree of fatty infiltration with persistent cell necrosis. *Case No. 29*—S-52-153. (H. & E. stain. ×175).

FIG. 8. Section of liver from a case of pulmonary tuberculosis showing the characteristic features of fatty infiltration, condensation of periportal connective tissue with inflammatory cell reaction. (H. & E. stain. ×175).

FIG. 9. Section of liver from a case of pulmonary tuberculosis showing more advanced cell destruction associated with mild fatty and inflammatory cell infiltration. *Case No. 69*—S-52-136. (H. & E. stain. ×365).

kept in mind with this periportal sclerosis, which in one case shows pseudolobular formation, it is difficult to dismiss easily the view expressed that pulmonary tuberculosis may lead to cirrhosis (figures 10 and 11). Schiff (18*d*) has shown by serial biopsy that sarcoidosis can lead to cirrhosis. Spink and co-workers (19) have also found the occurrence of cirrhosis in brucellosis. In the present study, one patient had actinomycotic infection of the lung, and the liver showed early diffuse fibrosis (figure 12). It is possible that granulomatous processes may be responsible in some instances for the production of diffuse hepatic fibrosis.

Infiltration with the inflammatory cells: Infiltration with chronic inflammatory cells, such as lymphocytes, plasma cells, and (in a few cases) neutrophils, was present in 9 untreated tuberculous patients. Saphir observed this type of change in almost all of his cases (figures 9 and 11). Similar cellular infiltrations were observed in the tissues of 8 tuberculous patients who had received some treat-

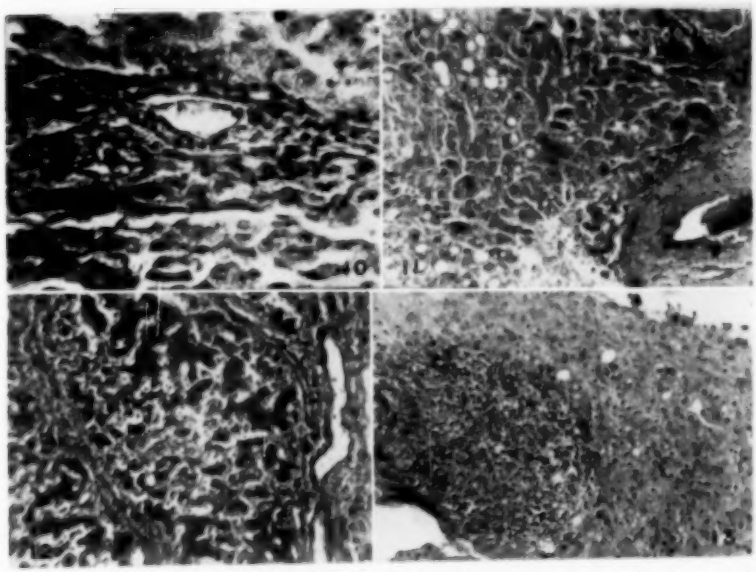

FIG. 10. Section of liver from a case of pulmonary tuberculosis showing the characteristic condensation of connective tissue in and around the portal tract, with inflammatory cell infiltration and bile duct proliferation. *Case No. 76*—S-52-38. (H. & E. stain. ×365).

FIG. 11. Section of liver from a case of pulmonary tuberculosis showing patchy fatty infiltration, marked condensation of periportal connective tissue which has become hyalinized. The condensed fibrous tissue which is seen at the periphery of the photograph is extended and joined with similar tissue from the neighboring lobule, giving the impression of diffuse hepatic fibrosis. *Case No. 64*—S-52-39. (H. & E. stain. ×175).

Fig. 12. Section of liver from a case of actinomycotic infection of the lung showing early diffuse fibrosis with cell degeneration and inflammatory cell infiltration. On the left-hand side of the picture is a portal tract showing proliferation of the bile ducts. Condensation of the connective tissue with inflammatory cell infiltration. *Case No. 29*—S-52-153. (H. & E. stain. ×175).

FIG. 13. Section of liver from a case of pulmonary tuberculosis showing focal collection of inflammatory cells unrelated to the portal tract, consisting of lymphocytes and plasma cells. Such collections have been interpreted as early stages in the evolution of a classical miliary tubercle. *Case No. 63*—S-52-196. (H. & E. stain. ×175).

ment. In 2 nontuberculous patients and in 3 tuberculous patients, there was a definite focal collection of mononuclear cells (figure 13).

Evolution of miliary tubercles: The biopsy material from 3 untreated and 3 streptomycin-treated patients revealed focal collections of mononuclear cells with pale epithelioid cells, but no Langhans' type of giant cell or caseation was present (figure 14). Definite classical miliary tubercles were present in 3 untreated and 2 treated patients from the tuberculous group (figures 15–19). Leurete (2*a*) and Saphir (11) found miliary tubercles in 99 and 80 per cent, respectively, of their patients. Examination of the post-mortem records in this

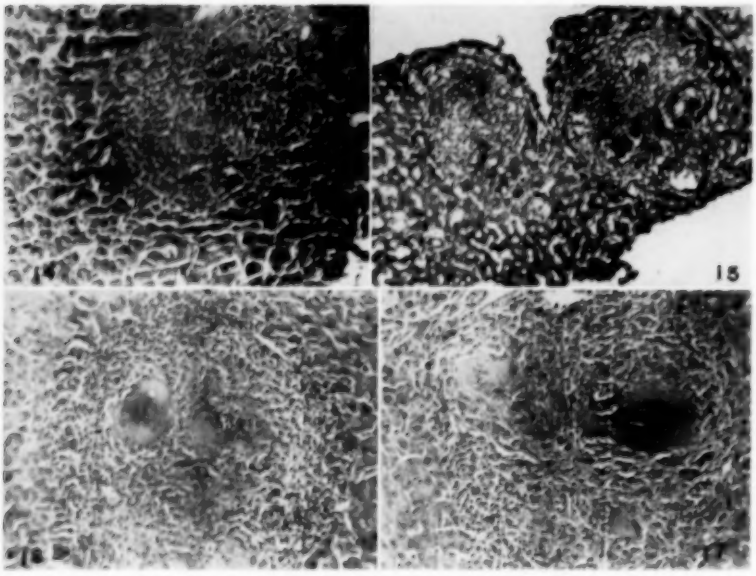

FIG. 14. Section of liver from a case of pulmonary tuberculosis showing focal collection of cells, unrelated to portal tract. The cells in the center of this collection are large, pale, with indistinct cell outline and are interpreted as epithelioid cells. *Case No. 79—S-52-108.* (H. & E. stain. ×175).

FIG. 15. Section of liver from a case of pulmonary tuberculosis showing a classical miliary tubercle with an adjoining focal collection of cells which is interpreted as an early tubercle. The sinusoids of the liver are widely dilated and are infiltrated with inflammatory cells. (H. & E. stain. ×175).

FIG. 16. Section of liver from a case of pulmonary tuberculosis showing the classical histology of a miliary tubercle. *Case No. 42—S-52-463.* (H. & E. stain. ×365).

FIG. 17. Section of liver from a case of pulmonary tuberculosis showing the presence of a classical miliary tubercle in the area of the portal tract with marked increase of both connective tissue element and inflammatory cells in the portal tract. *Case No. 42—S-52-463.* (H. & E. stain, ×175).

institution of 6 patients who had died from pulmonary tuberculosis showed that the incidence of miliary tubercles in the liver was 100 per cent. The failure to identify miliary tubercles in needle biopsy specimens may be explained by the obvious limitations of the technique as a true sampling device or possibly by the obscuring of the tubercle by such changes as cloudy swelling or fatty infiltration (20).

The incidence of miliary tubercles is much higher in patients having associated intestinal tuberculosis. This fact was very well illustrated by Saphir's investigations of one hundred patients with pulmonary tuberculosis, of which eighty had tubercles in the liver. Of these eighty patients, fifty-seven had associated intestinal tuberculosis.

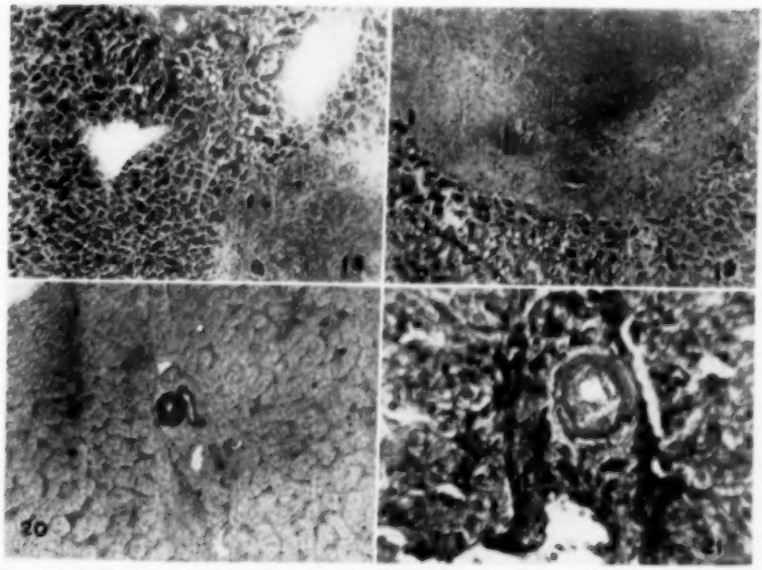

FIG. 18. Section of liver from a case of pulmonary tuberculosis showing at one end a tubercle which has almost replaced the whole of a hepatic lobule and adjoining lobule with dilated central vein and sinusoids, with patchy infiltration at the periphery of the lobule. Condensation of the periportal connective tissue is visible. (H. & E. stain. ×175).

FIG. 19. Section of liver from a patient with pulmonary tuberculosis who died and at post mortem examination revealed active tuberculous caseous pneumonia, showing massive areas of caseation in the liver with patchy fatty infiltration and dilatation of sinusoids infiltrated with inflammatory cells. (H. & E. stain. ×175).

FIG. 20. Section of liver from a case of pulmonary tuberculosis showing classical amyloid infiltration in the subintimal layer of the hepatic artery. *Case No. 53—S. 52 525.* (H. & E. stain. ×85).

FIG. 21. The section is the same as the one immediately above, stained for amyloid by methyl violet. (H. & E. stain. ×365).

Amyloid deposits: Amyloid deposits were present in one patient in the untreated pulmonary tuberculosis series (figures 20 and 21).

Relation of Abnormal Values for Hepatic Function and Histopathologic Findings

Structural changes were found in 6 patients in the nontuberculous series. Three of these patients had normal hepatic tests, while one showed abnormal values in two tests and the remaining 2 showed abnormal values in four tests. Six of the 35 nontuberculous patients showed normal function tests, one showed two abnormal, and another showed four abnormal, tests; yet all of these patients had completely normal hepatic tissue, as determined by examination of biopsy material. Of the 26 patients whose tissue removed at biopsy showed some type of abnormality, 20 had four, 5 had three, and one had two different types of liver function tests with abnormal values.

Six of the 19 patients in the streptomycin-treated group had normal histology. One of the group had normal values for all of the tests of hepatic function. Three of the remainder had two different abnormal functional tests, and 2 patients had three different abnormal tests. Thirteen patients showed some type of histologic abnormality in the biopsy material. In 9 of these patients, four different tests of hepatic function were abnormal; in 3 patients, three tests were abnormal; and, in 2 patients, abnormalities of function were detected in two tests.

All of the 6 patients in the nondrug-treated group had histologic abnormalities. Three of the 6 had four tests of liver function which were abnormal, 2 patients had three abnormal tests, and one patient had two abnormal tests.

There appeared to be a fairly close correlation between the structural changes, as determined by examination of the biopsy material, and the results of the tests of hepatic function. By the same token, function and structural abnormalities were independently present.

DISCUSSION

The present study has brought out very well that, in nontuberculous pulmonary infections, hepatic damage appears to be incidental and minor in degree. The structural changes in the liver in pulmonary tuberculosis, whether treated or untreated, were of greater magnitude and the incidence was much higher and striking than in the nontuberculous pulmonary infections.

It is reasonable to assume that the structural changes observed in the liver of patients with pulmonary tuberculosis can be reasonably attributed to the tuberculosis. The miliary tubercles and the focal collection of the mononuclear cells with pale epithelioid cells are presumably a direct result of the presence of tubercle bacilli. It is surprising that 5 of 60 patients had classical miliary tubercles in the liver without any evidence of generalized miliary tuberculosis on clinical examination. An additional 7 patients had tubercle-like lesions in the liver, again with no clinical manifestations of miliary tuberculosis.

The other changes, such as fatty infiltration, periportal increase in connective tissue, necrosis, and regeneration of the liver cells, may be the result of several causes. The most likely causative agents in the present study would appear to be nutritional factors related to the toxicity of the tuberculosis.

In experimental animals, nutritional factors may result in fatty infiltration, which may be intracellular as well as extracellular. Glynn and associates (18*a*) have shown that impairment of circulation takes place through the sinusoids of the liver which are being compressed by the liver cells that are distended with fat. In laboratory animals the nutritional cirrhosis is always central and of the "diffuse hepatic fibrosis" type.

In humans with nutritional edemas, Goplan and associates (21) and Higginson and co-workers (22) have reported necrosis of the liver cells, regeneration of the liver cells, and periportal fibrosis. They could not find any fatty infiltration, quite opposite to what has been observed in experimental animals as reported by Glynn (18*a*). As the fibrosis was periportal and not central, as observed in

the experimental animals, it seems that the mechanism by which cirrhosis (nutritional) takes place in humans in the early stages is not well understood. Zelman (23) investigated 19 obese patients and demonstrated by liver biopsy, fatty infiltration, necrosis, and periportal fibrosis a finding very similar to malnutrition. Zelman is of the opinion that this hepatic damage is due to relative hypoproteinemia, although protein fractionation was within normal limits.

In the present study functional and structural changes in the liver were investigated in other chronic pulmonary infections to serve as a basis for comparison. The environmental background of the patients was comparable, as most of them came from villages, from a low-income group, and, moreover, were suffering from chronic pulmonary infection. It was therefore expected that, if malnutrition were responsible for the hepatic damage in pulmonary tuberculosis, similar results would be found in the group with nontuberculous pulmonary infections.

In the present study, the evaluation of nutritional status of the patients was made on the basis of the following facts:

State of nutrition at the time of study as assessed clinically: *poor*—loss of subcutaneous fat, presence of edema; *average*—subcutaneous fat present, absence of edema; *good*.

Diet: vegetarian or nonvegetarian. No great significance can be attached to this factor as it not only depends upon the amount but also on the frequency of the animal proteins taken. As most of the patients belonged to the low-income groups, meat, fish, eggs, and milk were beyond the economic means, although the patients might have been nonvegetarians on other grounds.

The mean average of the serum proteins in the nontuberculous group, as well as in the patients showing normal and morbid histology in both groups.

The values for the serum albumin.

A comparative study of protein fractionation in relation to morbid and normal histology is presented in figure 22 and table 3.

The table illustrates that the nutritional states of the patients at the time of the present investigation did not have any significant relationship to the hepatic damage. Patients classified as having poor nutrition were actually fewer in the tuberculous group with morbid changes in the liver. Much importance cannot be attached to the dietetic habits of the patients, but the distribution is in no way greater in the group showing structural changes in the liver. The amount of serum proteins and albumin was definitely altered in the nontuberculous series with morbid changes in the liver. Probably in this group the hepatic damage was due to malnutrition, as there was an appreciable decrease in serum proteins and albumin, as illustrated in figure 22. There was a definite decrease in the mean averages of the serum proteins and albumin in the untreated pulmonary tuberculosis group. If figure 22 is scrutinized, however, it becomes evident that there was no significant difference in the serum protein in patients with normal or morbid changes in the liver in untreated pulmonary tuberculosis.

These protein changes can be either the result or the cause of hepatic damage. Seibert and Nelson (7) have shown by electrophoresis that, in experimental

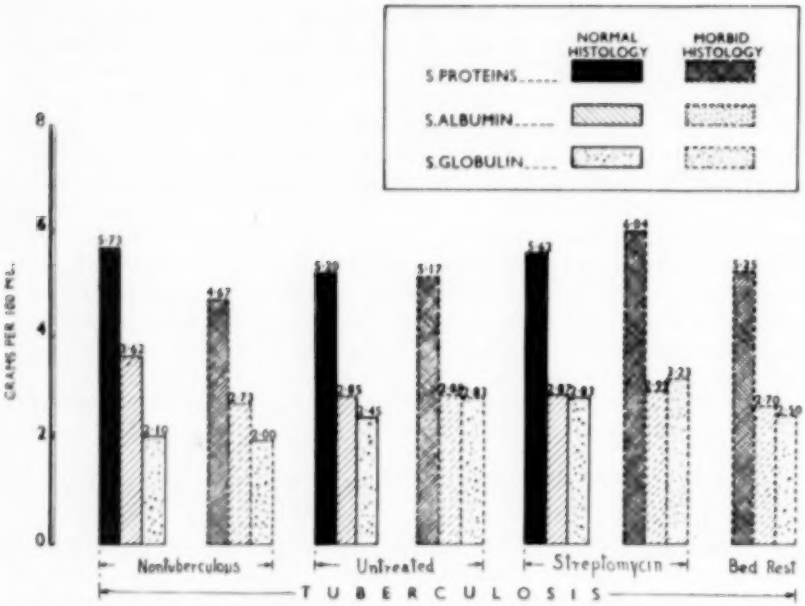

FIG. 22. Graph illustrating the results of protein fractionation in patients with normal and abnormal hepatic histology, as determined by examination of biopsy material.

animals as well as in forty-three patients suffering from pulmonary tuberculosis, a quantitative and qualitative change takes place in the serum albumin. In the present series, the altered protein values were also present in patients who showed normal liver tissue on examination of biopsy material. It will perhaps be more reasonable to interpret such changes as the results of toxicity of the pulmonary tuberculosis.

In the patients who had untreated pulmonary tuberculosis and who showed normal or morbid histology in the liver, there was no appreciable difference with respect to the duration of the illness. The same opinion was held by Galen, Weiner, and Hartman (8) in a study of fifty-three patients based on two hepatic tests, although the patients studied by them had illness which lasted approximately four to five years. In all of the nontuberculous bronchiectatic cases of the present study, the liver did not reveal any morbid changes, although the duration of the disease was 120 weeks, as is evident from table 4.

A critical histologic study revealed the presence of fatty infiltration in approximately 30 per cent of the patients in contrast to the situation in patients with nutritional edemas in whom fatty infiltration of the liver is either absent or minimal, as shown by Goplan and associates (21) and Higginson and co-workers (22). The increased condensation of the connective tissue was periportal in all of the cases, although, in experimental animals with nutritionally induced hepatic changes, the fibrosis is always central and diffuse. Necrosis and regenera-

TABLE 3

RELATIONSHIP OF HEPATIC STRUCTURAL DAMAGE TO NUTRITION, DIET, SERUM PROTEIN, AND SERUM ALBUMIN

Histology	Nontuberculous		Tuberculosis					
			Untreated		Streptomycin		Bed Rest	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Nutrition								
Poor	11	4	5	7	2	4	—	1
Average		2	—	16	3	8	—	4
Good	6	—	3	3	1	1	—	1
Diet								
Vegetarian	13	3	4	12	3	4	—	4
Nonvegetarian	15	2	2	14	3	9	—	2
Serum protein	5.73	4.67	5.2	5.17	5.60	6.04	—	5.25
Serum albumin	3.62	2.73	2.85	2.88	2.87	2.99	—	2.70

TABLE 4

RELATIONSHIP OF HEPATIC STRUCTURAL DAMAGE TO DURATION OF DISEASE

	Total Number of Cases	Number Showing Normal Histology	Duration of Illness	Number Showing Abnormal Histology	Duration of Illness
			weeks		*weeks*
Miscellaneous	3	1	25	2	6
Pleurisy with effusion	8	8	25	—	—
Bronchiectasis	9	9	137	—	—
Lung abscess	13	10	188	3	11.3
Tuberculosis (untreated)	34	8	34.5	26	37
Tuberculosis (streptomycin-treated)	17	6	36	11	21.6
Tuberculosis (bed rest)	6	—	—	6	41

tion of the liver cells is common to pulmonary tuberculosis, nutritional edema, and obesity.

All of the above facts point out that the changes in the liver other than those due to the direct invasion by *Mycobacterium tuberculosis* are most likely the results of tuberculous toxicity rather than due to pure malnutrition. Similar views have been expressed by Galen, Weiner, and Hartman (8) as a passing remark that "inanition is not related to the altered hepatic function as recorded by bromsulfalein retention and cephalin cholesterol tests."

SUMMARY

Ninety-four cases of chronic pulmonary infections, tuberculous and nontuberculous, were studied.

In the control group of 34 nontuberculous patients, more than three liver functions were disturbed in 5 patients. Thirty-three successful liver biopsies revealed morbid anatomy of minor degree in 6 cases.

In a group of 60 patients with pulmonary tuberculosis, 45 had an abnormal

thymol turbidity; 45 had cephalin cholesterol which was abnormal; and bromsulfalein retention was abnormal in 51 patients, with disturbance in albumin-globulin ratio. Serum bilirubin and alkaline phosphatase were within normal limits.

Examination of needle biopsy material showed fatty degeneration, necrosis, regeneration of liver cells, periportal sclerosis, infiltration with chronic inflammatory cells, classical miliary tubercles, and amyloid deposits. One or more of these changes were observed in 45 of 59 patients in whom successful biopsies were performed.

A definite correlation between structural and functional changes in the liver seemed to exist.

Fatty infiltration, periportal sclerosis, necrosis, and regeneration of cells, and infiltration with chronic inflammatory cells are presumably the result of the toxicity of the pulmonary tuberculosis. It has been suggested that this toxicity might indirectly lead to cirrhotic changes in the liver in some instances. As such structural changes in the liver were absent in the nontuberculous group, it is probable that nutrition does not play any direct role in their production.

The hepatic damage did not bear any relation to the duration of the pulmonary tuberculosis.

SUMARIO

Las Lesiones Hepáticas en La Tuberculosis Pulmonar Crónica

Este estudio comprendió 94 casos de infecciones pulmonares crónicas, ya tuberculosas o no. En el grupo testigo de 34 sujetos no tuberculosos, estaban trastornadas más de tres funciones hepáticas en 5 enfermos. Treinta y tres biopsias hepáticas ejecutadas con éxito revelaron anatomía morbosa de menor cuantía en 6 casos. En un grupo de 60 tuberculosos pulmonares, hubo anomalía de la turbidez del timol en 45, de la floculación de cefalina-colesterina en 45 y de la retención de bromosulfaleína en 51. La bilirrubina y la fosfatasa alcalina del suero quedaban dentro de límites normales.

Una biopsia con aguja reveló degeneración adiposa, necrosis, regeneración de las células hepáticas, esclerosis periporta, infiltración por ceélulas inflamatorias crónicas, tubérculos granúlicos clásicos y depósitos amiloides en 45 de 59 biopsias ejecutadas con éxito.

Parece existir una correlnción bien definida entre las alteraciones anatómicas y las funcionales en el hígado.

La infiltración adiposa, la esclerosis periporta, la necrosis, la regeneración celular y la infiltración por células inflamatorias crónicas son consecuencia de la toxemin tuberculosa. Se ha apuntado que puede provenir cirrosis del hígado de dicha toxemia.

Como no existían tales alteraciones anatómicas en el grupo testigo, es probable que la nutrición no desempeñe papel alguno en su producción.

La lesión hepática no guardó la menor relación con la duración de la enfermedad en la tuberculosis pulmonar.

RESUME

Lésion du foie dans la tuberculose pulmonaire chronique

Quatre vingt quinze cas d'infections pulmonaires chroniques, tuberculeuses et non tuberculeuses, ont été étudiés. Dans le groupe témoin, comprenant 34 malades non tuberculeux, 5 des malades présentaient une altération de plus de trois fonctions du foie. Trente trois biopsies adéquates ont révélé la présence de lésions de degré mineur dans 6 cas. Parmi un groupe de 60 malades affectés de tuberculose pulmonaire, la réaction d'opacité au thymol

était anormale dans 45, cas la cholestérol-céphaline était anormale dans 45 cas et la rétention de la bromosulfaléine était anormale dans 51 cas. La bilirubine sérique et la phosphatase alcaline restaient dans les limites normales.

Une biopsie par ponction montrait la dégénérescence graisseuse, la nécrose, la régénération des cellules hépatiques, la, sclérose périportale, linfiltration har des cellules de réaction inflammatoire chronique, les tubercules miliaires classiques et des dépôts amyloïdes dans 45 des 59 biopsies adéquates.

Une corrélation définie entre les modifications fonctionnelles et la structure paraît exister.

L'infiltration graisseuse, la sclérose périportale, la nécrose ainsi que la régénération des cellules et l'infiltration par des cellules de réaction inflammatoire chronique sont imputables à la toxémie tuberculeuse. L'auteur considère que la toxémie tuberculeuse pourrait déterminer la cirrhose hépathique.

Comme les modifications de structure manquaient dans le groupe témoin, il est probable que la nutrition ne joue aucun rôle dans leur production Dans la tuberculose pulmonaire, l'altération hépatique était sans aucun rapport avec la durée de l'affection.

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RESULTS OF SCALENE NODE BIOPSY IN PATIENTS WITH PULMONARY CALCIFICATIONS[1]

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INTRODUCTION

The differential diagnosis and evaluation of intrathoracic calcifications is an important problem. This problem often presented itself to the physicians on the Medical Service of the Lackland Air Force Base Hospital in 1951 when they were called upon to evaluate new recruits whose chest roentgenograms showed calcification of sufficient size or number to require proof of stability for retention of the recruit in the Service. Many of these recruits were residents of the Middle West, and skin test results suggested that histoplasmosis was the predominant factor which led to the calcification. In November of 1951, colleagues of the writer had the opportunity, following a suggestion by Dr. B. L. Corley, to prove the diagnosis of sarcoidosis by the scalene node biopsy technique (1) in 3 patients who exhibited roentgenographic evidence of bilateral hilar adenopathy. It seemed reasonable that this technique might also prove useful in determining whether histoplasmosis remained active in otherwise asymptomatic patients who presented roentgenographic evidence of pulmonary calcification.

METHODS AND MATERIALS

From December, 1951, through February, 1953, 50 young adult male patients were admitted to the Lackland Air Force Base Hospital for evaluation of pulmonary calcifications. These patients were subjected to clinical evaluation, roentgenographic study, skin testing with histoplasmin, and a scalene node biopsy was performed on each patient. Active pulmonary or systemic histoplasmosis was not clinically suspected in any patient and most patients were asymptomatic. No patient had palpable supraclavicular nodes, and acid-fast organisms were never obtained from sputum or gastric washings. Forty-two patients had multiple, mostly miliary, parenchymal lesions and, of this group, 3 had one or more calcified hilar nodes. Five patients had only one or two paratracheal densities, and 3 patients had small densities suggestive of a primary tuberculous complex. Patients were skin tested with histoplasmin[3] solution of 1:1,000 dilution, coccidioidin solution of 1:100 dilution, and PPD (purified protein derivative) in first and second strength (table 1). During one period of several months, histoplasmin skin-testing material was not available. However, of 24 patients tested with histoplasmin, 20 patients (83 per cent) gave positive reactions. Of 49 patients tested with PPD, 20 patients (42 per cent) were positive. There was one coccidioidin reactor. Eighteen states were represented in the total group of 50 patients, but 26 of the 50 patients were residents of Illinois, Indiana, or Missouri (table 2), where histoplasmosis is known to be endemic.

The scalene node biopsies were done by members of the surgical service of the Lackland Air Force Base Hospital. The technique of Daniels was followed, and in most cases the

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TABLE 1

RESULTS OF SKIN TESTS

Positive Reactors to Histoplasmin and PPD

	Number of Patients Tested	Number of Positive Reactors	Per Cent
Histoplasmin	24	20	83
PPD	49	20	42

TABLE 2

RESIDENCE OF PATIENTS WITH PULMONARY CALCIFICATIONS

State	Number of Patients
Alabama	1
Arkansas	2
California	2
Georgia	1
Illinois	7
Indiana	8
Iowa	1
Kansas	2
Kentucky	2
Maryland	2
Michigan	3
Missouri	11
Nebraska	1
New Mexico	1
North Carolina	1
Ohio	1
Tennessee	1
Texas	3

TABLE 3

HISTOLOGIC AND MYCOLOGIC RESULTS OF BIOPSIED SCALENE NODES

Total number of nodes		50
Nodes cultured for fungi		29
Positive results		1
Histoplasma capsulatum	1	
Nodes studied histologically		46
Positive results		4
Positive chronic lymphadenitis with fibrosis	1	
Sarcoidosis	2	
Chronic inflammation	1	

biopsied specimen of lymphoid material was divided equally between two containers destined for the histopathology and mycology laboratories, respectively. The case record of *Case 1* emphasizes the importance of careful division of the specimen in the operating room before submitting it to the laboratory for study.

Of the entire group, histologic study was carried out on forty-six nodes, and twenty-nine nodes were submitted for fungus culture (table 3). The records are incomplete with respect to the number of nodes cultured for acid-fast organisms, but the number was small and no positive results were obtained.

RESULTS

Of the entire group of 50 patients, only five nodes yielded abnormal results by any method of study. The cases of these patients are briefly summarized below.

Case 1 (reported in detail elsewhere (2)) was that of an 18-year-old white male from Missouri who had no symptoms. His chest roentgenograms showed disseminated miliary calcifications and indefinite enlargement of the hilar nodes. All skin tests were negative except for histoplasmin, which gave a 1 plus reaction. A scalene node biopsy was performed and the pathologist received a very small node which showed no histologic abnormality. However, the mycologist received a larger separate node measuring more than 1 cm. in diameter, from which he was able to grow organisms with the morphologic and growth characteristics of *Histoplasma capsulatum*. The strain recovered was pathogenic for mice. This patient was in good health when last heard from, sixteen months after the procedure. By a curious coincidence, this was the first scalene node in the series which was cultured and also the only node from which significant bacteriologic results were obtained.

Case 2 was that of an 18-year-old white male from Indiana who had a chronic cough productive of yellow sputum. His chest roentgenogram showed multiple calcifications and a persistent left lower lobe infiltration, the nature of which could not be determined. All skin tests were negative except the histoplasmin, which gave a 3-plus reaction. The histologic report on his scalene node was "chronic lymphadenitis with fibrosis." No fungi could be grown from the node. It is probable that, coincident with presumably healed histoplasmosis, this patient had bronchiectasis with chronic pulmonary suppurative disease.

Case 3 was that of a 21-year-old white male from Missouri who was admitted with a mild and probably insignificant cough. His chest roentgenogram showed multiple miliary calcifications. His histoplasmin skin test was 3-plus, and the PPD test was negative. A complement fixation test for histoplasmosis, performed on serum, was negative. The scalene lymph node was reported to show chronic granulomatous inflammation with giant cells containing stellate inclusions. The histologic appearance was considered to resemble that of sarcoidosis. The slide was submitted to the Armed Forces Institute of Pathology and the consultants there concurred in this impression. Further clinical and laboratory study of this patient failed to reveal clinical evidence of sarcoid disease.

Case 4 was similar. The patient was a 26-year-old asymptomatic white male from Michigan. The chest roentgenogram showed multiple calcifications and the histoplasmin skin test gave a 2-plus reaction. The scalene node was diagnosed as consistent with sarcoidosis on histologic examination. The staff of the Armed Forces Institute of Pathology, although concurring in the description of a granulomatous inflammation, preferred to characterize it as compatible with, but not typical of, sarcoidosis. Neither of the nodes from these 2 patients was studied bacteriologically.

Case 5 was that of a 20-year-old white male from Maryland without symptoms, whose chest roentgenogram showed a density in the second left interspace measuring 1.5 cm. in diameter, with central calcification. Skin tests were negative except for histoplasmin, which was 2-plus. The patient's serum complement fixation test for histoplasmosis was negative. The left scalene node was reported to show chronic inflammation and reactive hyperplasia. No culture was done.

An additional patient was of considerable interest but was excluded from the series because no calcifications appeared in the chest roentgenogram. He was a

20-year-old white male recruit from Illinois who had no symptoms but was sent to the hospital because of a right apical infiltration on his first chest roentgenogram. This lesion remained stable for a period of two months. Efforts to culture acid-fast organisms from gastric washings were unsuccessful. His tuberculin test remained persistently negative, but his histoplasmin skin test was 2-plus. A complement fixation test for histoplasmosis was positive on serum. The scalene node taken from this patient was not studied histologically, but cultures for acid-fast organisms and fungi were negative. The patient was given the presumptive diagnosis of pulmonary histoplasmosis.

DISCUSSION

It is now well accepted that in many areas of the country, particularly in the watershed of the Mississippi River, miliary calcifications are the result of previous infection with *Histoplasma capsulatum* (3). In the southwestern states, previous infection with *Coccidioides immitis* may lead to calcified residua in the lung parenchyma. The pulmonary form of both histoplasmosis and coccidioidomycosis is usually benign, and the calcific deposits probably represent well-healed residua. The majority of the patients in the present study lived in the great midportion of the United States prior to entering the Air Force. Indeed, 52 per cent claimed residence in the states of Illinois, Indiana, and Missouri, where the endemicity of histoplasmosis is known to be high. This fact plus the relatively greater incidence of positive reactors to histoplasmin than to PPD (83 per cent versus 42 per cent) led to the belief that most of the calcifications in this group were due to previous infection with histoplasmosis.

The size, location, or number of calcifications did not correlate positively with either tuberculosis or histoplasmosis. It has been established that the initial pulmonary lesion of histoplasmosis may take almost any form and that the resulting calcifications may be single or multiple, and of varying size (4). Pulmonary calcifications are not commonly seen in patients with disseminated histoplasmosis. Indeed, most recent reviews of the literature do not give any quantitative data regarding the presence or absence of calcium deposits in the lungs of patients with the disseminated disease. Hodgson, Weed, and Clagett (5) found that, of 65 reported patients with proved involvement of the lung, 11 subjects had roentgenographic evidence of calcifications in the lungs.

As calcification of the residual lesions probably requires approximately two years, it would seem a fair assumption that dissemination of histoplasmosis occurs less than two years after the primary infection in most, although not necessarily in all, cases. Nevertheless, the patient of *Case 1* of the present study, despite the presence of calcified lesions, had a continuing histoplasmic liability because virulent *Histoplasma capsulatum* could be isolated in pure culture from one of his lymph nodes. Despite this liability, he has continued well and asymptomatic for at least an additional sixteen months. Shefts, Terrill, and Swindell (6) have described a similar patient, who presented multiple miliary pulmonary calcifications, without symptoms, and from whose scalene node virulent histoplasmic organisms were cultured.

The scalene node from the patient in *Case 2* was diagnosed as "chronic lymphadenitis with fibrosis." Since cultures of this node were negative for pathogenic fungi and since this patient had a chronic productive cough, it is quite possible that this histologic change was secondary to chronic suppurative disease of the lung. The results of the node from the patient in *Case 5*, which was diagnosed chronic inflammation with reactive hyperplasia, do not lend themselves to satisfactory interpretation since this patient was entirely asymptomatic and no bacteriologic studies were performed on the biopsy material.

Two patients (*Cases 3* and *4*) whose nodes were microscopically compatible with the diagnosis of sarcoid disease are of more interest because there was no reason to suspect such a diagnosis clinically. The present writer is not aware of any similar instance previously reported. It is possible that these patients were detected at a very early stage of sarcoidosis or that the biopsies were made during a clinical remission of the disease. However, the granulomatous inflammation produced by fungi, and specifically histoplasmosis, has been reported to be a source of confusion in diagnosis because of its similarity to the lesion of sarcoidosis. Reiman and Price (7) and Israel and associates (8) have pointed out that bacteriologic study may be necessary to demonstrate *Histoplasma* in a lesion which is histologically indistinguishable from sarcoidosis. Unfortunately no bacteriologic study was done on these nodes. The 2 patients resided in Missouri and Michigan, respectively, had miliary multiple pulmonary calcifications, and positive histoplasmin skin tests. It is believed, therefore, that histoplasmosis is more likely responsible for these histologic changes than sarcoidosis.

It is of interest that only 5 of the 50 patients studied yielded scalene node material which gave abnormal results. The available knowledge of histoplasmosis is too limited to supply a satisfactory prognostic interpretation, even with respect to the patient who was found to be harboring virulent organisms in a lymph node. If it be assumed, however, that at least two years had elapsed during which calcification took place following the initial infection and, if to that is added the sixteen months during which the patient was known to have been free of symptoms following the biopsy, his outlook appears reasonably favorable. This certainly does not preclude the possibility that his disease might disseminate at some future time, particularly in view of the fact that the involved lymph node lacked the presumably protective calcific shell present in the pulmonary parenchymal lesions. In *Cases 3* and *4*, it is possible that *Histoplasma* organisms might have been demonstrated had the culture studies been performed. Unfortunately, the study was incomplete in that only twenty-nine of the fifty nodes were submitted for cultural study.

It is believed that for the routine evaluation of patients, such as for military service, scalene node biopsy is probably not economical in terms of anticipated results. On the other hand, it is a very simple and innocuous procedure. In the hands of the surgical consultants, 83 biopsies had been performed by the completion of this study and no serious complications had occurred. One incisional abscess occurred, which required simple drainage after one week, and one patient returned after approximately three months complaining of a vague numbness

and paresthesia, which did not follow any anatomical pattern, along the midportion of his arm. This was probably not related to his biopsy procedure. Therefore, when clinically indicated and for the further long-term study of the natural history of histoplasmic infection, this procedure should prove to be very useful.

SUMMARY

Fifty consecutive male Air Force recruits with pulmonary calcifications, the majority of which were multiple miliary lesions, were subjected to clinical study and scalene node biopsy. The place of residence and skin test results indicated that the majority of these calcifications were probably of histoplasmic origin.

Abnormal results were obtained from 5 patients. From one scalene node, a virulent culture of *Histoplasma capsulatum* was obtained. Two nodes were histologically consistent with sarcoidosis. These nodes were not cultured for *Histoplasma*. Two nodes showed a nonspecific inflammatory change. The clinical significance of these results is discussed.

Because of the difficulty in prognostic interpretation of results in the relatively small incidence of positive results, scalene node biopsy is probably not an economical procedure for the routine evaluation of patients with pulmonary calcifications. When clinically indicated for diagnostic purposes and for the further study of the natural history of histoplasmic infection, it should prove to be a useful procedure.

SUMARIO

Resultados de la Biopsia de los Ganglios Escalenos en los Enfermos que Tienen Calcificaciones Pulmonares

Cincuenta reclutas varones consecutivos de la Fuerza Aérea de los E. U. A. que tenían calcificaciones pulmonares, la mayoría de las cuales eran lesiones granúlicas múltiples, fueron objeto de un estudio clínico y de biopsias de los ganglios escalenos. El lugar de residencia y los resultados de la cutirreacción indicaron que la mayoría de dichas calcificaciones reconocía origen histoplásmico.

Obtuviéronse resultados anómalos en 5 enfermos. De un ganglio escaleno, se obtuvo un cultivo virulento de *Histoplasma capsulatum*. Estos ganglios no fueron cultivados en busca de *Histoplasma*. Dos ganglios mostraron una alteración inflamatoria anespecífica. Discútese la importancia clínica de estos resultados.

Debido a lo difícil que es la interpretación pronóstica de los resultados a la luz de la incidencia relativamente baja de positivas, la biopsia de los ganglios escalenos no constituye probablemente un procedimiento económico para la justipreciación corriente de los enfermos que tienen calcificaciones pulmonares. No obstante, cuando se halla indicada clínicamente para fines de diagnóstico, y para el estudio ulterior de la historia natural de la infección histoplásica, debe resultar un instrumento útil.

RESUME

Résultats de la biopsie de ganglions prélevés au niveau des scalènes chez des malades présentant des calcifications pulmonaires

Une étude clinique et la biopsie de ganglions prélevés au niveau des scalènes ont été effectuées chez une série de recrues de l'armée de l'air présentant des calcifications pulmonaires dont la majorité était des lésions miliaires. Le lieu de la résidence et les tests cutanés indiquaient que ces calcifications pouvaient avoir une origine histoplasmique.

Des résultats anormaux ont été obtenus chez 5 des patients. Une culture virulente de *H. capsulatum* a été obtenue à partir de ces ganglions. Ceux-ci n'avaient pas été cultivés en vue de la recherche de l'*Histoplasma*. Deux ganglions montraient un processus inflammatoire non spécifique. La signification chimique de ces résultats est commentée.

En raison de la difficulté de l'interprétation pronostique des résultats, à cause de la fréquence relativement faible des résultats positifs, la biopsie des ganglions au niveau des scalènes ne semble pas représenter un procédé économique pour l'évaluation routinière des cas présentant des calcifications pulmonaires. Quand il s'agit d'obtenir un diagnostic clinique, et pour l'étude complémentaire de l'évolution naturelle de l'infection histoplasmique, elle paraît être un procédé utile.

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FURTHER EXPERIMENTS WITH COMBINATIONS OF PANCREATIN AND QUATERNARY AMMONIUM COMPOUNDS FOR CULTIVATION OF MYCOBACTERIUM TUBERCULOSIS[1]

ROLF SAXHOLM

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INTRODUCTION

The use of a mixture of pancreatic enzymes with the quaternary ammonium compound methylphenyl-dodecyl-trimethyl-ammonium-methosulfate (Desogen®) for the pretreatment of sputum specimens for cultivation of tubercle bacilli was reported in a previous paper (1). The results obtained with this method and modifications of it in comparison with the sodium hydroxide method in the routine examination of 3,818 sputum specimens are reported in the present paper.

Hirsch (2) suggested the use of benzalkonium chloride for the isolation of tubercle bacilli. In the course of the studies reported in the previous paper, a mixture of benzalkonium chloride and pancreatin had also been included, and was compared with the pancreatin-Desogen® (*D*) and sodium hydroxide methods throughout the greater parts of the two experimental series. Since the results of the pancreatin-benzalkonium chloride method appeared to be inferior to those obtained with the pancreatin-*D* method, they were not included in the previous paper, but will be presented in the present report.

The results of some other experiments with the pancreatin-*D* method and modifications thereof will also be discussed.

MATERIAL AND METHODS

Sputum specimens: The sputum specimens were selected at random among those received for examination for tubercle bacilli. The volumes of sputum (2 or 3 ml.) used were approximately double those used in the previously reported experiments.

Pancreatin: Only freshly prepared, Seitz-filtered solutions of pancreatin were used in preparing the reagents.

Pancreatin-D (1 per cent) mixture: Equal volumes of 2 per cent water solutions of pancreatin and of methylphenyl-dodecyl-trimethyl-ammonium-methosulfate (Desogen®) were mixed.

Pancreatin-D (1.5 per cent) mixture: Three per cent solutions of Desogen® were prepared by diluting a 10 per cent stock water solution with Clark and Lubs' borate buffer (pH 9) and mixed with equal volumes of 2 per cent pancreatin solutions in the same buffer.

Pancreatin-benzalkonium chloride (1 per cent) mixture: Equal volumes of 2 per cent water solutions of pancreatin and of benzalkonium chloride (a commercial product stated to meet the standard requirements of the American pharmacopoeia) were mixed.

Procedure

The pancreatin-benzalkonium chloride method which was used in connection with earlier studies was carried out as described for the pancreatin-*D* methods in the previous paper (1).

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TABLE 1

DISTRIBUTION OF 434 POSITIVE RESULTS OBTAINED BY CULTIVATION OF 2,575 SPUTUM SPECIMENS FOR M. TUBERCULOSIS

Treatment with a Pancreatin-*D* (1 Per Cent) Mixture Dissolved in Water Compared with Sodium Hydroxide Treatment

Hours at Room Temperature with PD (1 Per Cent)	Noncontaminated Specimens			Contaminated Specimens		Total Numbers of Specimens Positive for *M. tuberculosis*		
	Both Methods Positive	PD (1 Per Cent) Positive; NaOH Negative	PD (1 Per Cent) Negative; NaOH Positive	PD (1 Per Cent) Contaminated; NaOH Positive	PD (1 Per Cent) Positive; NaOH Contaminated	PD (1 Per Cent)	NaOH	Both Methods
19	227	62	43	27	5	294	297	364
44	38	16	10	6	0	54	54	70
Total	265	78	53	33	5	348	351	434

The treatment time with the pancreatin-*D* (1 per cent) method was approximately nineteen and forty-four hours.

PD = pancreatin-*D*

NaOH = sodium hydroxide

In the pancreatin-*D* methods, equal volumes of sputum and reagents were transferred to 13 mm. wide test tubes which were stoppered, shortly shaken by hand, and placed in a dark cupboard at room temperature. The average treatment time for the majority of samples was approximately nineteen hours. Samples received on Saturdays, however, were left with the reagent until Monday morning, corresponding to an average treatment time of forty-four hours.

The sodium hydroxide method was the same as in the previous study except that a phenol red solution instead of litmus paper was used as an indicator in the neutralization, which was carried out immediately after centrifugation.

Roughly the same quantity of inoculum, one to two drops from wide uncalibrated Pasteur pipettes, from the pancreatin *D*- and sodium hydroxide-treated samples was inoculated onto Löwenstein-Jensen slants, corresponding to the usual conditions in the routine work.

TABLE 2

THE CONTAMINATION RATES OF THE PANCREATIN-*D* (1 PER CENT) AND SODIUM HYDROXIDE METHODS IN 2,141 SPUTUM SPECIMENS NEGATIVE FOR M. TUBERCULOSIS

Hours at Room Temperature with PD (1 Per Cent)	Numbers and Percentages of Noncontaminated Specimens	Numbers and Percentages of Specimens with at Least 2 of 4 Slants Contaminated			Total
		PD (1 Per Cent) Only	NaOH Only	Both Methods	
19	1,608 (88.9%)	181 (10%)	12 (0.7%)	8 (0.4%)	1,809
44	293 (88.3%)	34 (10.2%)	4 (1.2%)	1 (0.3%)	332
Total	1,901 (88.8%)	215 (10%)	16 (0.8%)	9 (0.4%)	2,141

See table 1 for explanation of the abbreviations.

TABLE 3

DISTRIBUTION OF 209 POSITIVE RESULTS OBTAINED BY CULTIVATION OF 1,243 SPUTUM SPECIMENS FOR M. TUBERCULOSIS

Treatment with a Pancreatin-*D* (1.5 Per Cent) Mixture Dissolved in Borate Buffer of pH 9 Compared with Sodium Hydroxide Treatment

Hours at Room Temperature with PD (1.5 per cent)	Noncontaminated Specimens			Contaminated Specimens		Total Numbers of Specimens Positive for *M. Tuberculosis*		
	Both Methods Positive	PD (1.5 Per Cent) Positive; NaOH Negative	PD (1.5 Per Cent) Negative; NaOH Positive	PD (1.5 Per Cent) contaminated; NaOH Positive	PD (1.5 Per Cent) Positive; NaOH Contaminated	PD (1.5 Per Cent)	NaOH	Both Methods
19	100	43	32	2	3	146	134	180
44	18	6	5	0	0	24	23	29
Total	118	49	37	2	3	170	157	209

The treatment time with the pancreatin-*D* (1.5 per cent) method was approximately nineteen and forty-four hours.

See table 1 for explanation of abbreviations.

TABLE 4

THE CONTAMINATION RATES OF THE PANCREATIN-*D* (1.5 PER CENT) AND SODIUM HYDROXIDE METHODS IN 1,034 SPUTUM SPECIMENS NEGATIVE FOR M. TUBERCULOSIS

Hours at Room Temperature with PD (1.5 Per Cent)	Numbers and Percentages of Noncontaminated Specimens	Numbers and Percentages of Specimens with at Least 2 of 4 Slants Contaminated			Total
		PD (1.5 Per Cent) Only	NaOH Only	Both Methods	
19	869 (96.3%)	24 (2.7%)	8 (0.9%)	1 (0.1%)	902
44	130 (98.5%)	0	2 (1.5%)	0	132
Total	999 (96.6%)	24 (2.3%)	10 (1%)	1 (0.1%)	1,034

See table 1 for explanation of abbreviations.

Unlike the previous experiments, no attempt was made to standardize the size of inoculum to represent the same quantity of sputum by both methods.

The inoculated slants were examined for growth once a week from the third to the sixth weeks after inoculation.

Otherwise the methods were as previously described.

RESULTS

The pancreatin-*D* (1 per cent) and the sodium hydroxide methods are compared with respect to numbers of positive results and to contamination rates in tables 1 and 2, and the pancreatin-*D* (1.5 per cent) and the sodium hydroxide methods are similarly compared in tables 3 and 4. Samples treated for approximately nineteen and forty-four hours are shown separately in the tables.

Only specimens which showed a sufficiently heavy contamination to prevent growth of tubercle bacilli on two or more of the four slants have been counted as contaminated, since a minor degree of contamination was not considered sufficient to reduce the chance of obtaining positive results.

Virtually the same numbers of positive cultures for tubercle bacilli were obtained by the pancreatin-*D* (1 per cent) and by the sodium hydroxide methods on the 2,575 samples shown in table 1. This was attained in spite of an over-all contamination rate of 9.6 per cent for the pancreatin-*D* (1 per cent) method and 0.8 per cent for the sodium hydroxide method in the 2,575 specimens. Of the 248 specimens classified as contaminated with the pancreatin-*D* (1 per cent) method, all four culture slants were overgrown by contaminants in 175, three slants in 60, and two slants in 13 cases. Thus, the contamination was sufficiently heavy in these cases to eliminate or greatly reduce the chance of obtaining a positive result.

Consequently, in the group of contaminated specimens (table 1), the sodium hydroxide method produced an excess of 28 positive results. On the other hand, the pancreatin-*D* (1 per cent) method produced an excess of 25 positive results in the group of noncontaminated specimens. These specimens in the latter group, which gave a positive result by the pancreatin-*D* (1 per cent) method only, mostly gave growth of few colonies of tubercle bacilli. This indicated that the method more easily demonstrates the presence of small numbers of tubercle bacilli than is the situation with the sodium hydroxide method. The positive samples lost by the pancreatin-*D* (1 per cent) method through contamination included many specimens which produced heavy growth of tubercle bacilli by the sodium hydroxide method.

A series of 1,243 specimens was examined by the pancreatin-*D* (1.5 per cent) technique, which resulted in a reduction of the over-all contamination rate from 9.6 per cent of the pancreatin-*D* (1 per cent) technique to 2.1 per cent. The contamination rate of the sodium hydroxide method in the 1,243 specimens was 1.0 per cent. Concurrently, more samples were positive for tubercle bacilli by the pancreatin-*D* (1.5 per cent) method than by the sodium hydroxide method. Using the mathematical formulas given in the previous paper, the difference between the frequencies of positive results by the two methods amounts to 1.164 times the standard error of this difference, corresponding to a probability level of 24.4 per cent. Thus, the difference is not statistically significant.

Experiments to be reported in detail later indicate that the pancreatin-*D* method works well with even higher concentrations of Desogen®; for example, 2 and 3 per cent. A certain concentration of Desogen® is necessary to obtain satisfactory results, but an increase of the concentration beyond such a level does not cause any significant reduction of the contamination rate, nor does it, on the other hand, appear to reduce the number of positive results to any great extent.

The pancreatin-*D* method appeared to work equally well with a treatment time of forty-four hours instead of nineteen hours. The effect of prolongation of the treatment time has been studied in special experiments, and it appears that the pancreatin-*D* treatment exerts a mild effect, even over longer periods of time.

TABLE 5

COMPARISON OF THE GROWTH RATE OF TUBERCLE BACILLI FROM SPUTUM AFTER TREATMENT WITH PANCREATIN-*D* (1.5 PER CENT) AND WITH SODIUM HYDROXIDE

The Specimens Which Were Positive by Both Methods Have Been Arranged According to the Time Intervals in Weeks Between the Appearance of Colonies by the Two Methods

Methods	PD (1.5 Per Cent)			Both Methods	NaOH			Total
Antecedence of growth in weeks	3	2	1	0 = simultaneous growth appearance	1	2	3	
Distribution of specimens	3	3	28	81	3			118

See table 1 for explanation of the abbreviations.

TABLE 6

CULTIVATION OF M. TUBERCULOSIS FROM SPUTUM AFTER TREATMENT WITH PANCREATIN-*D* (1 PER CENT), PANCREATIN-BENZALKONIUM CHLORIDE (1 PER CENT), AND SODIUM HYDROXIDE, RESPECTIVELY

Hours at Room Temperature with PD (1 Per Cent) and PB (1 Per Cent)	Total Numbers of Specimens	Specimens Positive for *M. Tuberculosis*		
		PD (1 Per Cent)	PB (1 Per Cent)	NaOH
4	344	60	36	55
24	228	56	30	36

PD = pancreatin-*D*
PB = pancreatin-benzalkonium chloride
NaOH = sodium hydroxide

All of the comparative experiments between the pancreatin-*D* and the sodium hydroxide methods have left the definite impression that growth appears earlier with the pancreatin-*D* method. This is supported by the data shown in table 5, in which specimens positive by both the pancreatin-*D* (1.5 per cent) and the sodium hydroxide methods are grouped according to the time intervals in weeks between the recording of the positive results after the alternative treatments.

Fewer positive results were obtained when benzalkonium chloride was substituted for Desogen®. These data are presented in table 6, in which the numbers of positive results after treatment with pancreatin-benzalkonium chloride (1 per cent), pancreatin-*D* (1 per cent), and sodium hydroxide, respectively, are compared. These sputum specimens were all included in the previously reported experimental series. The same trend has been indicated in other series, using different concentrations, shorter treatment time, and also incubation at 37° C. Approximately the same contamination rate was found as with Desogen®.

As pointed out in the previous work, different quaternary ammonium compounds have been tried and some of these have appeared very promising as follows:

> β-phenoxyaethyl-dimethyl-dodecyl-ammonium-bromide (Bradosol,® Ciba, Switzerland) and, if used with borate buffer (pH 9), cetyltrimethylammonium bromide (Cetavlon,® "improved quality", Imperial Chemical (Pharmaceuticals) Ltd., England).

Other quaternary compounds have appeared to be less suitable for this purpose (dodecanoyl-N′-methylaminoethyl)-(phenyl-carbamyl-methyl)-dimethylammonium-chloride (Desogen® in dry substance, Geigy, Switzerland), dodecyl-ethoxyamide pyridinium sulfate (AM4, Sopura, Belgium), dodecyl-triphenyl-phosphonium-bromide (Karl Thomae, Germany), cetyl pyridinium chloride (Pyrisept,® Weifa, Norway), *p*-diisobutylphenoxyethoxy-ethyldimethyl-benzylammonium chloride (Phemerol,® Parke, Davis & Co., Ltd., England, and hyamine® 1622, Rohm and Haas, Co., U.S.A.), and di-n-decyl-dimethylammonium bromide (Deciquam® 222, The British Hydrological Corporation, England).

Papain (3) has been used instead of pancreatin and has been buffered to a pH within its optimal range. It is very serviceable, but the pancreatin preparation used seems to have a stronger digestive power. Pepsin has also been used. It was used at an appropriate pH in combination with cetyl pyridinium chloride, which was reported by Quisno and Foter (4) to maintain its bactericidal activity at a relatively low pH. This method also seems promising.

The pH of the pancreatin-*D* mixture was measured to 8.5 when a borate buffer solution of pH 9 was used as solvent for the ingredients, and to 4.5 when water was used. The latter pH was unexpectedly low and was shown to be due to the pancreatin solution. An adequate homogenization effect was, however, attained at pH 4.5, although, as expected, it seemed to be better at pH 8.5.

The pancreatin-*D* method has also worked well with the blood medium of Tarshis (5), but not with the Dubos Tween®-albumin agar medium.

In a great number of cases, guinea pig inoculations have been carried out parallel to the pancreatin-*D* and sodium hydroxide culture methods. The results indicate that growth of acid-fast saprophytes which could be mistaken for tubercle bacilli has been very rare and equally rare by both culture methods.

DISCUSSION

It may be pointed out that the results of the two series of observations reported in this paper corroborate each other in demonstrating that the pancreatin-*D* reagents are comparatively innocuous to the tubercle bacilli. The pancreatin-*D* (1 per cent) would have given superior results to the sodium hydroxide method except for the loss through contamination.

The contamination rate in the present series was unexpectedly high, compared with the previously reported results as well as with the results of later experimental series, which have shown consistently low contamination rates. The cause of this discrepancy is unknown, but two differences in the technique used in this study in comparison with that used in the other experimental series may possibly be of significance.

First, larger volumes of sputum, but the same 13 mm. wide tubes, were used in the series reported in this paper. Since the pancreatin-*D* mixture, in spite of a short shaking by hand, tends to form a layer above the tenacious sputum and only slowly mixes with the latter, the smaller contact area per volume may have caused a delay of the enzyme action and, at the same time, of the action of the decontaminant. Second, larger inocula have been used in these series than in other experimental series. This leads to a larger quantity of enzyme, which may cause a softening of the medium. It seems possible that this may favor the spread

of contaminants on the surface of the slants, whereas on drier media a few contaminating organisms may remain as isolated colonies. Experiments are under way to study the significance of these factors.

The success of the method would depend upon the possibility of reducing the contamination rate without reducing the yield of positive results. The composition of the pancreatin-*D* (1 per cent) mixture was therefore changed by increasing the concentration of Desogen® to 1.5 per cent, maintaining the concentration of pancreatin at 1 per cent. In view of the fact that pancreatin as well as many of the quaternary ammonium compounds show a higher degree of activity at an alkaline reaction, the reagents were dissolved in borate buffer at pH 9. The favorable outcome of these changes is reported above.

It has not yet been possible to reproduce quite as favorable results with the pancreatin-*D* method, compared with the sodium hydroxide method, as those presented in the previous report. Nevertheless, in all series which have been concluded so far, the pancreatin-*D* results have balanced, or been better than, those of the sodium hydroxide method. Various possible factors in addition to those mentioned are being studied. The low contamination rate of the most favorable series with the pancreatin-*D* method in comparison with the sodium hydroxide method in the previous report is noteworthy.

The pancreatin-*D* (1.5 per cent) method has now been used as a routine method in this laboratory for several months. The sputum specimens are generally received in the British "universal containers," and the reagent is introduced directly into these containers. It may be an advantage that the latter are wider than the test tubes in the above-reported series, since a comparatively greater contact area between sputum sample and reagent is provided. Experience has shown that the amount of labor saved by using this method is very considerable, and it is believed that hazards of laboratory infection are less than with the sodium hydroxide method, as suggested previously (1).

The fact that neither the concentration of Desogen® nor treatment time is a critical factor adds to the practical applicability of the method. Moreover, this may open the possibility of adding the pancreatin-*D* mixture to the specimens at the places of collection before sending them to the laboratory. Thus, the treatment of the sputum specimens could be started as early as possible and the multiplication of contaminants prevented during the transport. The shaking of the containers, which will occur during the transport, should only hasten the digestion and decontamination of the specimens. They would be received in the laboratory ready for inoculation. It may be recalled that Mitchell, Jefferies, and Stucker (6) advocated the addition of trisodium phosphate to the specimens at the places of collection.

A great difference has been found between the different quaternary compounds with respect to germicidal effect on both tubercle bacilli and contaminants. A proper balance is an essential requirement. It would seem probable that still more selective compounds than those examined might be found. A study of additional compounds is being undertaken, and additional selective derivatives might perhaps become available.

It might be pointed out that cetyl pyridinium chloride in combinations with pepsin and pancreatin, respectively, at different pH levels did not seem to give equally serviceable results.

The inferior results obtained by the pancreatin-*D* method with Dubos Tween®-albumin agar medium may be due to the failure of this semisynthetic medium to neutralize or combine with the quaternary ammonium compounds, in contrast to media which contain more organic matter. This neutralizing capacity is believed to be connected with phospholipids (7). Addition of such a substance to Dubos agar or to the inoculum might perhaps make the medium serviceable for this method.

Results of experiments on the use of the pancreatin-*D* method with the slide culture technique will be published elsewhere (8). The applicability of the method on other kinds of specimens is being studied.

A detailed report of these and other experiments will be published in the near future.

SUMMARY

Three thousand and eight hundred and eighteen sputum specimens were digested and decontaminated for cultivation of tubercle bacilli by means of mixtures of pancreatin and the quaternary ammonium compound Desogen®. The method compares favorably with the sodium hydroxide method. Results obtained with different combinations of proteolytic enzymes and quaternary ammonium compounds, as well as with different media, are reported.

SUMARIO

Nuevos Experimentos con Combinaciones de Pancreatina y Compuestos Cuaternarios de Amonio para el Cultivo del M. Tuberculosis

Tres mil ochocientos dieciocho ejemplares de esputo fueron digeridos y descontaminados para el cultivo del bacilo tuberculoso por medio de mezclas del compuesto cuaternario de amonio, desógeno, con pancreatina. El método se compara favorablemente con el del hidróxido de sodio. Preséntanse los resultados obtenidos con distintas combinaciones de enzimas proteolíticas y compuestos cuaternarios de amonio, así como con diversos medios.

RESUME

Nouvelles recherches sur la culture du M. tuberculosis à l'aide combinaisons de composés d'ammonium quaternaire et de pancréatin

La digestion et la purification préalables de trois mille huit cent dix huit spécimens de crachats ont été pratiquées à l'aide d'un mélange de dosogen, composé d'ammonium quaternaire avec la pancréatine. Cette méthode soutient favorablement la comparison avec le procédé à l'hydroxyde de sodium. Les auteurs rapportent les résultats obtenus avec différents combinaisons d'enzyme protéolytiques et deux composés d'ammonium quaternaire, ainsi qu'avec différents milieux.

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THE PROBLEM OF THE TUBERCULOUS PSYCHOTIC[1]

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INTRODUCTION

Numerous surveys conducted during the past two decades (1–6) have furnished adequate factual proof of the old observation that tuberculosis is an important problem among hospitalized psychotics. Under the impact of these surveys, chest roentgenography on the admission of the patients to the hospital and at regular intervals during their residence has become a routine procedure of progressive mental hospitals.

The present tuberculosis control program of the U. S. Veterans Administration initiated under General Hawley (7) and further described by Wolford (8) and Schneider and Robins (9) was initiated at the Veterans Administration Hospital, Downey, Illinois, in 1946. Under this program all patients received a chest roentgenogram on their admission. Patients found to have or suspected of having tuberculosis were segregated on the tuberculosis unit. Here they underwent the usual diagnostic procedures, including laboratory examination and bronchoscopy when needed, and received appropriate treatment. The unit was attached to the large tuberculosis service of the general hospital and was under the direction of its chief of service. The final diagnosis was arrived at and the treatment prescribed by the staff of this Tuberculosis Service. A chest roentgenogram was an integral part of the periodic annual physical examination and was the responsibility of the ward physician. Chest roentgenograms were also taken on other occasions, particularly when a patient developed a respiratory ailment, low-grade fever, loss of weight, or other sign of illness. Irrespective of the date of such occasional roentgenograms, the survey films were taken annually. Whenever a case of tuberculosis was discovered, all contacts, both patients and personnel on the ward, were examined roentgenographically, irrespective of the date of previous examinations.

Prior to the inauguration of the present control program in 1946, routine annual roentgenograms of the resident patients were not obtained. However, almost 80 per cent of the patients admitted to this hospital prior to 1946 had had chest films at the time of their admission, as it was customary to obtain a film at the time of a patient's admission and whenever indicated because of clinical symptoms or tuberculous contact during his hospital residence. Moreover, a tuberculosis survey had been conducted at this hospital in 1936–1937 (5) during which a chest roentgenogram was obtained on every patient and, in many instances, the procedure was repeated. As a result of these early efforts to control tuberculosis, most of the patients in the Veterans Administration Hospital at Downey at the start of the present control program in 1946 had received sporadic roentgenographic examinations during the period of their hospitalization prior to 1946.

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The present study is an attempt to determine the magnitude of the tuberculosis problem in the Veterans Administration Hospital at Downey, Illinois. Particular effort has been made to evaluate the prevalence of active tuberculosis among patients admitted for treatment of a mental disorder and the prevalence and incidence of active tuberculosis among hospitalized psychotic patients as well as the effect of shock therapy on the incidence of tuberculosis.

METHOD OF STUDY

The medical records of every mental patient hospitalized at the hospital at any time between January 1, 1946, and December 31, 1950, were studied and the required data were transcribed onto an abstract form. The records of patients with a history of tuberculosis were then subjected to special study. All chest roentgenograms obtained in each case, irrespective of the date, were also reviewed and the findings recorded. In all, 19,935 roentgenograms were thus studied. In the few instances in which films were not available for the pre-study period, the clinical records were reviewed and the roentgenographic findings were accepted as given. *Active tuberculosis* was diagnosed when the sputum examination revealed tubercle bacilli, in the presence of characteristic changes in the serial roentgenograms, or upon autopsy findings. *Inactive tuberculosis* was diagnosed on the basis of the characteristic roentgenographic findings, with or without a history of a previously active disease.

The mental diagnoses were grouped under the three main headings of *functional psychoses*, *organic psychoses*, and *other neuropsychiatric disorders*. The original data were classified into twelve subcategories, but the numbers in these categories were too small to be handled meaningfully.

There were comparatively few females and nonwhites in the study group. Likewise, there were few nonpsychotic patients hospitalized at any one time. For these reasons the analysis of prevalence and incidence of tuberculosis among mental patients dealt only with male white psychotic patients except for the prevalence of tuberculosis among admissions which was calculated for all male white mental patients. Patients admitted to this hospital by transfer from other Veterans Administration installations were not included in the computations of prevalence of tuberculosis among admissions. For, the Downey Veterans Administration Hospital has facilities for the treatment of tuberculous psychotics and, consequently, patients are transferred there for utilization of this service. However, patients transferred to Downey Veterans Administration Hospital for treatment of a psychosis without concurrent tuberculosis are included in the prevalence and incidence rates among the resident psychotic patients.

Incidence rates were computed on the basis of person-years of exposure. The person-years of exposure were calculated by adding the time in years that each patient was under observation. In order to be included in the study of incidence, a patient had to have had a normal roentgenogram at this hospital after January 1, 1946, and at least one subsequent roentgenogram during his hospitalization. The date of the patient's last normal or first abnormal roentgenogram was considered as the termination of his observation period.

OBSERVATIONS

Prevalence of Tuberculosis Among Patients Admitted to the Hospital

There were 1,076 male white patients admitted to Downey Veterans Administration Hospital for treatment of a mental disorder from January 1, 1946, to December 31, 1950. Of these 1,076 patients, 10 (0.9 per cent) were found to have active pulmonary tuberculosis. The tuberculosis prevalence rates according to psychiatric diagnosis and age groups are given in table 1. The prevalence rate among those patients more than forty-five years of age was 1.9 per cent; while, for patients less than forty-five, it was only 0.7 per cent.

TABLE 1

NUMBER OF UNSUSPECTED, ACTIVE PULMONARY TUBERCULOSIS CASES AND PREVALENCE RATES PER 100 MALE, WHITE PATIENTS ADMITTED DIRECTLY TO A MENTAL INSTITUTION DURING THE PERIOD FROM JANUARY 1, 1946, THROUGH DECEMBER 31, 1950, BY TYPE OF DISORDER AND AGE*

Age	Type of Mental Disorder											
	All Types			Functional Psychosis			Organic Psychosis			Other Mental Disorders		
	Total Admissions	Cases of Active Pulmonary Tuberculosis		Total Admissions	Cases of Active Pulmonary Tuberculosis		Total Admissions	Cases of Active Pulmonary Tuberculosis		Total Admissions	Cases of Active Pulmonary Tuberculosis	
		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent
All ages	1,076	10	0.9	512	3	0.6	119	1	0.8	445	6	1.3
Less than 25	170	0	0.0	104	0	0.0	5	0	0.0	61	0	0.0
25 to 34	488	4	0.8	251	2	0.8	28	0	0.0	209	2	1.0
35 to 44	212	2	0.9	89	0	0.0	29	0	0.0	94	2	2.1
45 to 54	135	3	2.2	50	1	2.0	28	0	0.0	57	2	3.5
55 to 64	58	1	1.7	17	0	0.0	19	1	5.3	22	0	0.0
65 and older	13	0	0.0	1	0	0.0	10	0	0.0	2	0	0.0

* None of the patients included in this table was known to have active tuberculosis at the time of admission and none had been known to have previously had inactive tuberculosis.

It should be pointed out that in many cases the diagnosis was made in retrospect when serial roentgenograms and laboratory results were available. Hence, corrections could be made for oversights and a definite diagnosis established in suspected cases. In some cases, the diagnosis was established only after repeated roentgenograms. It was found that such checks and rechecks were apt to increase the number of cases discovered, particularly among psychotics, because their inability to cooperate often leads to technically unsatisfactory films.

The over-all tuberculosis prevalence rate of 0.9 per cent found among persons admitted to the hospital during the period 1946 through 1950 was much higher than prevalence rates found in surveys of the general population. However, other studies (9–11) have indicated rates of 0.4 to 0.8 per cent among routine admissions to general hospitals and even higher rates (12) for other specific disease categories. Since, as was indicated above, the prevalence rate as found at this hospital was believed to be a little high because of retrospective diagnoses, there seems no reason to conclude that the prevalence of tuberculosis among persons admitted for treatment of a mental condition is any higher than among any other group of sick persons. Moreover, the majority of the patients admitted to the hospital came from the metropolitan area of Cook County, Illinois, where the tuberculosis morbidity is relatively high.

The prevalence rate of tuberculosis was slightly higher among those patients admitted for a psychoneurotic or a character and behavior disorder than it was among patients admitted for the treatment of a psychosis. Unfortunately, the length of hospitalization for patients with a psychoneurosis or character and

behavior disorder was relatively short, and thus it was not possible to determine an incidence rate of tuberculosis for this group of patients.

Prevalence of Unsuspected Tuberculosis Among Resident Patients

There were 1,744 male white psychotics hospitalized on January 1, 1946. Of this group, 43 patients were being treated for active pulmonary tuberculosis. Of the 1,701 male white psychotics without known active tuberculosis, 37 (2.2 per cent) were found to have active tuberculosis when they were first examined roentgenographically under the newly initiated case-finding program.

In the strictest sense, the rate as presented is not a true prevalence rate because a period of months was required to obtain chest roentgenograms on all of the patients. Thus, the 2.2 per cent of the psychotic patients found to have active tuberculosis on the first roentgenogram obtained after the official start of the revitalized case-finding program represents a slight overstatement of the number of unsuspected active cases of tuberculosis which existed among the psychotic patient load on January 1, 1946. Nevertheless, it is an accurate representation of the number of tuberculous patients found on the first mass survey of the hospital since 1937.

As may be seen in table 2, the prevalence of tuberculosis is much higher in the older age groups. Among psychotics forty-five years or older, the rate was 2.6 per cent while, among those patients less than forty-five, the rate was only 0.6 per cent. Among the functional psychotics, the prevalence rate of active tuberculosis was 2.3 per cent while, among those patients suffering from organic psychoses, the rate was 2.0 per cent. This difference existed for all age groups

TABLE 2

NUMBER OF UNSUSPECTED, ACTIVE PULMONARY TUBERCULOSIS CASES AND PREVALENCE RATES PER 100 MALE, WHITE, PSYCHOTIC PATIENTS HOSPITALIZED, BY TYPE OF PSYCHOSIS AND AGE*

Age	Type of Psychosis								
	All Psychoses			Functional Psychosis			Organic Psychosis		
	Total Persons†	Cases of Active Pulmonary Tuberculosis		Total Persons†	Cases of Active Pulmonary Tuberculosis		Total Persons†	Cases of Active Pulmonary Tuberculosis	
		Number	Per Cent		Number	Per Cent		Number	Per Cent
All ages	1,702	37	2.2	1,244	28	2.3	458	9	2.0
Less than 25	51	0	0.0	51	0	0.0	0	0	0.0
25 to 34	199	1	0.5	195	1	0.5	4	0	0.0
35 to 44	89	1	1.1	78	1	1.3	11	0	0.0
45 to 54	851	23	2.7	598	18	3.0	253	5	2.0
55 to 64	450	10	2.2	301	8	2.7	149	2	1.3
65 and older	62	2	3.2	21	0	0.0	41	2	4.9

* Some of the patients resident in the hospital on January 1, 1946, were not examined roentgenographically until 1947.

† Excludes psychotic patients known to have active tuberculosis.

except those patients sixty-five and older, among whom the prevalence rate was higher for persons with an organic psychosis than it was for functional psychotics. It is believed, however, that the difference between the two diagnostic groups was too small to be significant. Even though the prevalence rate for schizophrenic patients at Downey Hospital was found to be 2.5 per cent or higher than for the functional psychotic group as a whole, there was little evidence from this study to support the theory that there is some etiologic association between tuberculosis and schizophrenia (13).

The seemingly wide difference between the prevalence of tuberculosis among new hospital admissions of 0.9 per cent and the prevalence rate of 2.2 per cent found on a survey of the resident population was caused in large part by the different age distributions of the two groups. Actually, for psychotic patients less than forty-five, the prevalence rate of admissions was 0.4 per cent, based on 2 cases of active tuberculosis among 506 psychotic admissions, compared with a rate of 0.6 per cent found among 338 resident patients as of January 31, 1946. Among those psychotic patients more than forty-five years of age, the prevalence rate for newly admitted patients was 1.6 per cent (2 of 125), compared with a rate of 2.6 per cent based on 35 cases of tuberculosis found among 1,363 resident patients at the hospital on January 1, 1946. In the statistical sense, there is no significance in the difference in the prevalence rate of tuberculosis between the newly admitted and the resident patient groups.

Incidence

The incidence rates of tuberculosis are presented in tables 3 and 4. The over-all incidence of active tuberculosis was 8.2 per 1,000 person-years. There was, however, a great difference in incidence between patients with inactive pulmonary tuberculosis and patients with no history of tuberculosis. The latter group had

TABLE 3

INCIDENCE RATES FROM JANUARY, 1946, TO JUNE, 1951, OF ACTIVE PULMONARY TUBERCULOSIS AMONG MALE, WHITE, PSYCHOTIC PATIENTS WITH NO PREVIOUS HISTORY OF TUBERCULOSIS, BY TYPE OF PSYCHOSIS AND AGE

Age at Start of Observation	Type of Psychosis											
	All Psychoses				Functional Psychosis				Organic Psychosis			
	Number of Persons	Person-Years of Exposure	Active Pulmonary Tuberculosis Cases	Incidence Rate per 1,000 Person-Years	Number of Persons	Person-Years of Exposure	Active Pulmonary Tuberculosis Cases	Incidence Rate per 1,000 Person-Years	Number of Persons	Person-Years of Exposure	Active Pulmonary Tuberculosis Cases	Incidence Rate per 1,000 Person-Years
All ages	2,558	6,333	36	5.7	2,097	4,941	30	6.1	461	1,392	6	4.3
Less than 25	236	337	1	3.0	233	335	1	3.0	3	2	0	0.0
25 to 34	772	1,213	10	8.2	762	1,198	10	8.4	10	15	0	0.0
35 to 44	292	520	2	3.8	274	491	2	4.1	18	29	0	0.0
45 to 54	756	2,610	14	5.4	523	1,819	12	6.6	233	791	2	2.5
55 to 64	438	1,499	8	5.3	291	1,056	5	4.7	147	443	3	6.8
65 and older	64	154	1	6.5	14	42	0	0.0	50	112	1	8.9

TABLE 4

INCIDENCE RATES OF ACTIVE PULMONARY TUBERCULOSIS AMONG MALE, WHITE, PSYCHOTIC PATIENTS WITH INACTIVE TUBERCULOSIS, HOSPITALIZED DURING THE PERIOD JANUARY, 1946, TO JUNE, 1951, BY TYPE OF PSYCHOSIS AND AGE

Age at Start of Observation	Type of Psychosis											
	All Psychoses				Functional Psychosis				Organic Psychosis			
	Number of Persons	Person-Years of Exposure	Active Pulmonary Tuberculosis Cases	Incidence Rate per 1,000 Person-Years	Number of Persons	Person-Years of Exposure	Active Pulmonary Tuberculosis Cases	Incidence Rate per 1,000 Person-Years	Number of Persons	Person-Years of Exposure	Active Pulmonary Tuberculosis Cases	Incidence Rate per 1,000 Person-Years
All ages	179	578	18	31.1	127	406	14	34.5	52	172	4	23.3
Less than 25	0	0	0	—	0	0	0	—	0	0	0	—
25 to 34	6	8	1	125.0	6	8	1	125.0	0	0	0	—
35 to 44	8	25	0	0.0	7	21	0	0.0	1	4	0	0.0
45 to 54	101	341	11	32.3	72	243	9	37.0	29	98	2	20.4
55 to 64	55	175	6	34.3	41	129	4	31.0	14	46	2	43.5
65 and older	9	29	0	0.0	1	5	0	0.0	8	24	0	0.0

an incidence rate of 5.7 per 1,000 person-years while, among patients with a history of tuberculosis, the reactivation rate was 31.1 per 1,000 person-years. This difference clearly indicates the need for a close check of mental patients who have inactive tuberculosis.

The incidence of active tuberculosis was slightly higher among patients suffering from functional psychosis than it was among those patients with an organic psychosis. Among patients more than fifty-five years of age, however, this relationship was reversed and the incidence of active tuberculosis was higher among patients hospitalized for an organic psychosis.

The age group from twenty-five to thirty-four had the highest incidence rate of tuberculosis. The patients in this age group were veterans of World War II and were admitted to the hospital just prior to, or during, the study period. Since most of the tuberculosis that developed among this group developed during the first two years of the patients' hospitalization, consideration might be given to a program of semiannual chest roentgenograms for the first two years following a patient's admission.

Shock Therapy

The incidence rate of active tuberculosis among patients who had received shock therapy was 6.0 per 1,000 persons per year and, among those with no shock, it was 6.2 per 1,000 persons per year. These figures refer only to those patients with no history of tuberculosis. No cases of active tuberculosis developed among patients with inactive or arrested tuberculosis who received shock therapy, but this group was very small.

Because of the low incidence of active tuberculosis among patients in the study group, it was not feasible to evaluate the individual effect of the different types of shock therapy, nor was it feasible to evaluate the relationship between

TABLE 5

INCIDENCE RATES OF ACTIVE PULMONARY TUBERCULOSIS AMONG MALE WHITE PATIENTS WITH A FUNCTIONAL PSYCHOSIS HAVING NO HISTORY OF PRIOR TUBERCULOSIS HOSPITALIZED DURING THE PERIOD JANUARY, 1946, TO JUNE, 1951, BY AGE AND ACCORDING TO SHOCK THERAPY STATUS

Age at Start of Observation	No Shock Therapy				Shock Therapy			
	Number of Patients*	Patient-Years of Exposure	Active Pulmonary Tuberculosis Cases	Incidence Rate per 1,000 Person-Years	Number of Patients	Patient-Years of Exposure†	Active Pulmonary Tuberculosis Cases	Incidence Rate per 1,000 Person-Years
All ages	1,378	2,925	18	6.2	1,010	2,016	12	6.0
Less than 25	117	38	0	0.0	167	297	1	3.4
25 to 34	352	154	2	13.0	557	1,044	8	7.7
35 to 44	153	167	0	0.0	170	324	2	6.2
45 to 54	468	1,563	11	7.0	85	256	1	3.9
55 to 64	275	964	5	5.2	29	92	0	0.0
65 and older	13	39	0	0.0	2	3	0	0.0

* Includes persons who were observed before receiving shock therapy.

† Includes only periods of observation after shock therapy.

the incidence of tuberculosis and the elapsed time after shock. Moreover, as the reason for the selection of patients for shock therapy might be related to the patients' general condition, it is difficult to reach any clear-cut conclusion on the effect of shock therapy. There was no evidence, however, that shock therapy is contraindicated for psychotic patients because of the possibility of an activation or reactivation of tuberculosis.

DISCUSSION

The patients included in the present study are veterans. As veterans, the patients had to meet, at one time in their lives, the physical requirements imposed by the Armed Forces, and should, therefore, be considered a selected group. There are other factors which make comparisons with other investigations difficult. As previously mentioned, the tuberculosis prevalence rates presented were higher than those obtained by means of a single chest roentgenographic survey, since the patients were under constant observation and retrospective judgments were possible. Likewise, the incidence rates were higher than incidence rates computed from general statistics morbidity data. This presumably reflects the fact that surveillance of a patient population will result in the detection of cases of minimal active pulmonary tuberculosis which under ordinary circumstances would clear up spontaneously and only be discovered in the inactive stage (14).

For example, cases of inactive tuberculosis were discovered on the initial survey of the hospital in 1946 among patients who had been in residence for some years and whose admission films obtained at some previous time had revealed no evidence of tuberculosis. It must be assumed that these patients experienced an episode of active pulmonary tuberculosis while in residence without

the condition ever having been diagnosed. During the study period, however, with an intensive roentgenographic program in effect, it is doubtful that any patient with this type of minimal tuberculosis escaped detection during the active phase of the disease.

A comparison of the data from the present study with the experience of other groups of investigators is difficult. Not only are there differences in patient composition, criteria for diagnosis, and case-finding procedures, but the relatively few cases of active tuberculosis found in the present study make it impossible to correlate the incidence and prevalence of tuberculosis with the different types of mental illness. For example, it was not possible to make a positive statement about the relationship of tuberculosis to schizophrenia except, as was previously mentioned, to indicate that, if a relationship did exist, it was not marked enough to be demonstrable in a group of this size. The same sort of statement may be made concerning the environmental effects of hospitalization on the prevalence of tuberculosis. While the prevalence of active tuberculosis was slightly higher in the initial survey of the hospitalized psychotic population than it was among psychotic admissions, the difference was too small to be significant, especially when the difference in incapacitation between the groups is taken into account. The fact that the incidence of tuberculosis was lower in those patients forty-five years of age and older than it was in the younger patients, most of whom were admitted subsequent to World War II, seemed to indicate that extended hospitalization was not responsible for any appreciable increase in the attack rate of tuberculosis among the psychotic patients.

Since in a group this size the incidence rates were not appreciably affected by the type of psychosis or increasing age, there would not seem to be any reason to use these criteria in the operation of a tuberculosis control program. The wide difference between the incidence rates of active tuberculosis for psychotics with previously inactive tuberculosis and psychotics with no history of tuberculosis was dramatic enough, however, to indicate a need for closer supervision and more frequent roentgenograms among the group with inactive disease. It is suggested that these patients have roentgenographic examinations every three months instead of the customary yearly examination.

The over-all incidence rate of 5.7 cases of active tuberculosis per 1,000 person-years of exposure among psychotics with no history of tuberculosis is not believed to be excessive (15, 16), especially since some of the patients had capricious eating habits and many lacked hygienic motivation. The tuberculosis case-finding program was probably effective in holding down the incidence of tuberculosis, as indicated by the fact that 23 of the 36 cases of tuberculosis (64 per cent) which developed in the study group represented minimal lesions. Notwithstanding this finding, one-sixth of the patients were discovered to be tuberculous only after the appearance of clinical symptoms. This shows the fallacy of relying solely and entirely on the annual roentgenographic surveys. The continued awareness of the attending staff in regard to tuberculosis is just as essential to effective tuberculosis control as is the annual roentgenogram. This awareness, as a rule, is present to a higher degree in institutions where the annual chest roentgenographic survey has become a routine procedure than it is in institu-

tions with no program. Hence, the discovery of tuberculosis by clinical symptoms in the group of patients in the present study does not detract from the effectiveness of the survey program, but rather adds to it.

The crude death rate from tuberculosis during the study period was less than 2 per 1,000 per year, and tuberculosis accounted for less than 8 per cent of the total deaths at the hospital. This proportionate morbidity ratio was limited to only those cases of tuberculosis which developed among mental patients during their hospitalization. Since there were numerically so few tuberculous deaths, it was not practical to compute age-specific death rates, but it would appear that, in general, tuberculosis morbidity has been decreasing as the result of energetic treatment by modern methods, including surgery and chemotherapy. In addition, this intensive treatment will probably lower the incidence of tuberculosis since, by treating the patient, protection is afforded to all who come in contact with him.

SUMMARY

The prevalence of unsuspected, active tuberculosis among male white patients admitted to a mental institution was not significantly different from prevalence rates obtained in investigation of patients admitted to general hospitals.

The prevalence of tuberculosis among male white mental patients was much higher among patients more than forty-five years of age than it was among patients between twenty and forty-five years of age.

The incidence of active tuberculosis among male white psychotic patients with no history of tuberculosis was 5.7 per 1,000 person-years. Among male white psychotic patients with previously inactive or arrested tuberculosis, the rate was 31.1 per 1,000 person-years. There is an obvious need for close surveillance of those patients with inactive or arrested tuberculosis.

Despite an active case-finding program, one-sixth of the tuberculosis cases discovered among the psychotic patients were detected only after the appearance of clinical symptoms.

No evidence was found to indicate that use of shock therapy resulted in an increase in the incidence of tuberculosis.

SUMARIO

El Problema del Psicótico Tuberculoso

La incidencia de tuberculosis activa insospechada entre los varones blancos ingresados en un frenocomio no discrepó mayor cosa de los coeficientes obtenidos en las investigaciones de ingresos en los hospitales generales.

La frecuencia de la tuberculosis entre los enfermos mentales blancos del sexo masculino fué mucho mayor entre los enfermos de más de cuarenta y cinco años de edad que entre los de veinte a cuarenta y cinco años de edad.

La incidencia de tuberculosis activa entre los psicóticos blancos sin historia de tuberculosis fué de 5.7 por 1,000 personas-años. Entre los psicóticos varones blancos que habían tenido antes tuberculosis inactiva o estacionada, el coeficiente fué de 31.1 por 1,000 personas-años. Se necesita a las claras cuidadosa vigilancia de los enfermos que tienen tuberculosis inactiva o estacionada.

A pesar de un plan activo de descubrimiento de casos, la sexta parte de los casos de tuberculosis descubiertos entre los psicóticos no fué descubierta sino después de la aparición de síntomas clínicos.

No se observó contraindicación alguna del uso del choque terapéutico.

RESUME

Le problème de la tuberculose chez le malade mental

La prédominance de la tuberculose inapparente en activité chez des blancs de sexe masculin, hospitalisés dans les établissements pour maladies mentales, ne présentait pas de différence significative par rapport aux taux de prédominance obtenu dans les hopitaux pour maladies générales.

Dans la race blanche, la prédominance de la tuberculose chez les hommes atteints de maladie mentale était plus élevée parmi ceux qui avaient dépassé quarante-cinq ans que dans le groupe de vingt à quarante-cinq ans.

L'incidence de la tuberculose en activité chez les malades mentaux de race blanche sans antécédents de tuberculose était 5,7 pour 1000 hommes-années. Parmi les malades mentaux de race blanche avec antécédents de tuberculose inactivée ou stabilisée, le taux était de 31,1 pour 1000 hommes-années. La nécessité d'une surveillance étroite chez ces malades dont la tuberculose est inactivée ou stabilisée est évidente.

En dépit d'un programme actif de dépistage de la tuberculose, un sixième des cas de tuberculose découverts chez ces malades mentaux n'a été décelé qu'après apparition que signes cliniques.

Aucune contre-indication à l'électrochoc n'a été découverte.

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Notes

EFFECT OF CYCLOSERINE ON EXPERIMENTAL TUBERCULOSIS IN GUINEA PIGS

Cycloserine, a newly described antimicrobial produced by *Streptomyces orchidaceus*, has a wide antibacterial activity[1]. It inhibits growth of *M. tuberculosis in vitro* in a concentration of 10 γ per ml. of medium[2] and has been reported to be an effective agent in the treatment of pulmonary tuberculosis in man[3]. In contrast, three different groups of investigators have shown cycloserine to be inactive in mouse tuberculosis[1]. This apparent paradox has prompted the writers to study the effect of the drug on experimental tuberculosis in guinea pigs. The present paper is a preliminary report on experimental findings which will be described subsequently in more detail.

TABLE 1

EFFECT OF CYCLOSERINE, AS COMPARED WITH ISONIAZID, ON EXPERIMENTAL TUBERCULOSIS IN GUINEA PIGS

Drug	Dosage (mg./kg./day)	Weight Change (Average)	Average Index of Tuberculosis*
		gm.	
—	—	−6	10.8
Isoniazid	37.5	+123	0.2
Cycloserine	37.5	+56	11.8
	75.0	+46	9.8
	150.0	+51	8.5

* Maximum possible, 16.

Sixty guinea pigs with an average weight of 400 gm. were infected subcutaneously in the right inguinal region with 0.1 mg. of the H37Rv strain of virulent human tubercle bacilli. Seven days after infection they were divided into five groups. One group of 20 animals served as untreated controls, while the other four groups of 10 animals each were treated with either isoniazid or cycloserine, according to the dosage schedule presented in table 1. Both drugs were administered intramuscularly in three divided doses per day, alternating between the right and left thighs. Cycloserine solutions were prepared fresh daily in a pH 7.4 phosphate buffer. All animals, including the untreated controls, were weighed once a week during the treatment period. After sixty days of treatment, all of the surviving animals were killed. Each organ (lungs, liver, spleen, lymph nodes) was assigned a value of 0 to 4 according to the amount of gross tuberculosis seen[4].

It will be seen from the results presented in table 1 that cycloserine is relatively inactive against experimental tuberculosis in guinea pigs. There is evidence of a very slight therapeutic effect at the higher dosage levels, but this is certainly by no means as marked as that produced by isoniazid.

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The fact that cycloserine, despite its tuberculostatic effect *in vitro*, is relatively inactive against tuberculosis in the guinea pig might be attributable to (*1*) rapid excretion of drug, (*2*) conversion to inactive material in the host tissue, (*3*) slow diffusion from the site of injection, or (*4*) extremely rapid emergence of drug-resistant strains of bacilli. It is interesting to note, in this regard, that the tuberculostatic effect *of* cycloserine *in vitro* is slightly less pronounced in the presence of 5 per cent guinea pig serum than it is in the basal medium alone or in basal medium plus 5 per cent human serum[2].

In summary: Under the experimental conditions described, cycloserine appears to be relatively inactive against tuberculosis in guinea pigs as compared with isoniazid.

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CHROMOGENIC ACID-FAST BACILLI FROM HUMAN SOURCES

IV. *In Vitro* Response to a Number of Antimicrobial Agents on Glycerol-Blood Agar Medium

In previous papers,[1] cultural, pathologic, and hypersensitivity studies on a group of chromogenic acid-fast bacilli isolated from patients with, or suspected of having, tuberculosis were reported. Although the problem of the pathogenic potential of atypical mycobacteria is controversial, there is evidence that some of these strains can produce disease in man.[1, 2] Since the question has been raised many times regarding the antimicrobial agents which might possibly be effective against some of these organisms, it was believed advisable to investigate this aspect of the problem. The present preliminary investigation concerns the *in vitro* response of some of these strains to a number of such agents. The sources and general bacteriologic characteristics of the organisms have been described previously.[1]

The tests were performed by the indirect method on glycerol-blood agar medium,[3, 4] using plain agar instead of blood agar base. As this medium was found satisfactory for determining the susceptibility of tubercle bacilli to streptomycin and isoniazid, it was considered worth while to ascertain whether it could also be used to determine the susceptibility of the chromogenic acid-fast bacilli not only to these agents but to a number of others. The formula and method of preparation were as follows:

Glycerol-blood agar medium:

Plain agar (Difco)[5]	1.5 gm.
Glycerol (analytical grade)	1.0 ml.
Human bank blood (containing ACD solution, *see* below)	15.0 ml.
Water (distilled)	84.0 ml.
Final pH approximately 7.1	

Preparation: The medium was prepared in Erlenmeyer flasks by dissolving the agar in the glycerol-water by heating. The mixture was sterilized by autoclaving at fifteen pounds of pressure (121°C.) for fifteen minutes, after which it was cooled to 45°C. in a water bath. The blood and desired concentrations of the antimicrobial agents were added to the flasks and the ingredients were mixed thoroughly. Another flask of medium without any of the test agents was prepared for a control. The medium was dispensed aseptically into sterile screw-cap tubes and allowed to harden into slants. The flasks were kept in beakers of water in the bath to prevent tipping when the medium became depleted during the tubing process and were shaken frequently to maintain thorough mixture. Outdated bank-blood approximately four weeks old was used and contained ACD solution as follows: citric acid, 0.50 gm.; sodium citrate, 1.37 gm.; and dextrose, 2.45 gm., per each 100 ml. of transfusion solution.[6] To obviate possible inhibitory effects of any single blood, although such a problem has not been recognized, several specimens of the same type were pooled. The media were tested for sterility before use.

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[5] Difco Laboratories, Inc., Detroit, Michigan. Control No. 393371.

[6] Approximately 500 ml. of blood were collected in Baxter Transfuso Vac bottles containing 120 ml. of ACD solution. Baxter Laboratories, Inc., Morton Grove, Illinois.

TABLE 1

RESULTS OF ANTIMICROBIAL SUSCEPTIBILITY DETERMINATIONS ON GLYCEROL-BLOOD AGAR MEDIUM OF TEN STRAINS OF CHROMOGENIC ACID-FAST BACILLI

Response to Single Agents

Strain	C	SM, γ per ml.			PAS, γ per ml.			VM, γ per ml.			OT, γ per ml.			CT, γ per ml.			P, Units per ml.			I, γ per ml.		
		1	10	100	1	10	100	1	10	100	1	10	100	1	10	100	1	10	100	1	10	100
S-3	3+	3+	2+	0	0	0	0	3+	0	0	3+	3+	2+	3+	3+	2+	3+	3+	3+	3+	3+	3+
S-7	3+	3+	1+	3c	1+	1+	10c	2+	1+	0	3+	3+	2+	3+	2+	2+	3+	3+	3+	3+	3+	3+
S-8	3+	2+	1+	1+	3+	2+	2+	2+	1+	0	3+	1+	0	3+	2+	1+	3+	3+	3+	3+	3+	3+
M-1	3+	3+	2c	0	3+	8c	4c	2+	40c	0	2+	2+	2+	3+	2+	2+	2+	2+	2+	3+	3+	3+
M-6	3+	1+	4c	0	1+	5c	1c	3+	2+	0	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
W-1	3+	2+	0	0	2+	2+	2+	3+	2+	0	3+	3+	2+	3+	3+	3+	3+	3+	3+	3+	3+	3+
W-3	3+	3+	0	0	0	0	0	3+	2+	0	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
W-7	3+	0	0	0	0	0	0	5c	0	0	3+	3+	2+	3+	3+	3+	3+	3+	3+	3+	3+	3+
509	3+	2+	2+	2+	3+	3+	3+	3+	2+	2+	3+	3+	3+	2+	2+	1+	3+	3+	3+	3+	3+	3+
886	3+	3+	1+	0	3+	3+	3+	3+	2+	1+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

Symbols 1+ to 3+ represent final degree of growth after twenty-eight days at 37°C. *See* text.

C indicates control; SM, streptomycin sulfate; PAS, sodium para-aminosalicylate; VM, viomycin sulfate; OT, oxytetracycline (Terramycin® hydrochloride); CT, chlortetracycline (Aureomycin® hydrochloride); P, potassium penicillin G; I, isoniazid.

All the organisms were grown on Löwenstein-Jensen medium[7] for two weeks and then subcultured in the following manner to Tween®-albumin medium[8] to obtain dispersed growth. To ensure representative sampling, the inoculating loop was moved carefully through the entire culture, and then three heavy loopfuls of the organism were inoculated into the liquid medium. The cultures were incubated for two weeks at 37°C., and then adjusted to the No. 5 tube of the McFarland nephelometer standard[9] (equivalent to approximately 1.5×10^9 bacterial units per ml.) with Tween®-albumin medium. Five-hundredths milliliter inocula (containing approximately 7.5×10^7 bacterial units) were arbitrarily selected for study. All diluted cultures were checked for purity by smears and subculture into brain heart infusion and thioglycollate broth. The control tubes and those containing the antimicrobial agents were then inoculated and placed into the incubator in a nearly horizontal position to permit uniform distribution of the organisms. The cultures were incubated for twenty-eight days at 37°C., after which time readings were made. The final degree of growth was recorded as follows:

4-plus = surface of medium entirely covered
3-plus = growth not quite confluent
2-plus = innumerable colonies
1-plus = 50 to 200 colonies
Actual colony counts made if less than 50

Each experiment was repeated, and the final readings represent average results.

[7] TARSHIS, M. S., *et al.*: J. Bact., 1953, *66*, 448.

[8] DUBOS, R. J., AND MIDDLEBROOK, G.: Am. Rev. Tubere., 1947, *56*, 334.

[9] KOLMER, J. A., AND BOERNER, F.: Approved Laboratory Technic, ed. 4, D. Appleton-Century, New York and London, 1945.

TABLE 2

RESULTS OF ANTIMICROBIAL SUSCEPTIBILITY DETERMINATIONS ON GLYCEROL-BLOOD AGAR MEDIUM OF TEN STRAINS OF CHROMOGENIC ACID-FAST BACILLI

Strain	C	Response to Combined Agents											
		SM-PAS, γ per ml.			SM-VM, γ per ml.			PAS-VM, γ per ml.			SM-PAS-VM, γ per ml.		
		1	10	100	1	10	100	1	10	100	1	10	100
S-3	3+	3+	0	0	3+	0	0	2+	0	0	3+	0	0
S-7	3+	2+	2+	1+	3+	0	0	0	0	0	1+	0	0
S-8	3+	3+	0	0	3+	0	0	2+	0	0	2+	0	0
M-1	3+	3+	0	0	3+	0	0	0	0	0	1+	0	0
M-6	3+	0	0	0	3+	0	0	3+	0	0	3+	0	0
W-1	3+	2+	2+	0	3+	0	0	3+	0	0	3+	0	0
W-3	3+	1+	0	0	2+	0	0	2+	0	0	3+	0	0
W-7	3+	0	0	0	2+	0	0	0	0	0	0	0	0
509	3+	3+	2+	0	2+	0	0	2+	0	0	2+	0	0
886	3+	2+	0	0	Sc	0	0	2oc	0	0	0	0	0

Symbols 1+ to 3+ represent final degree of growth after 28 days at 37°C. *See* text.

C indicates control; SM, streptomycin sulfate; PAS, sodium para-aminosalicylate; VM, viomycin sulfate; 1, 10, and 100 γ represent final concentrations of each agent.

In the initial experiment, susceptibility determinations were performed with various concentrations of the following agents: streptomycin sulfate,[10a] sodium para-aminosalicylate,[11] isoniazid,[12] viomycin sulfate,[10b] oxytetracycline,[10c] chlortetracycline[13] and potassium penicillin G.[14] From the data in table 1, it can be seen that the greatest inhibitory capacity was exhibited by streptomycin, followed in descending order by para-aminosalicylate, viomycin, oxytetracycline, chlortetracycline, and penicillin. The inability of isoniazid to affect any of the organisms in any concentration is interesting in view of its efficacy against human tubercle bacilli. Although several strains (S-3, W-3, and W-7) were completely inhibited by the smallest concentrations of streptomycin or para-aminosalicylate, the over-all pattern of inhibition by the other agents was not striking.

Since the greatest inhibition was exhibited by streptomycin, para-aminosalicylate, and viomycin, it was believed advisable to ascertain whether a more pronounced inhibition would result by various combinations of these agents, each in the same concentrations used in the first experiment. From the data recorded in table 2, it will be noted that the inhibitory effect of the combined agents was very much more pronounced than that of the single agents.

The criteria of an ideal medium for performing susceptibility tests, the limitations inherent in such an *in vitro* procedure, and the various concentrations of blood which can be employed successfully have been discussed previously.[3] Although the blood

[10] Chas. Pfizer & Co., Inc., New York, N. Y. (*a*) Lot No. PKD521612; (*b*) Lot No. 24KF531150; (*c*) Terramycin® hydrochloride, Lot No. WKD537563.

[11] Merck & Co., Inc., Rahway, N. J. Lot No. 51131.

[12] Dinacrin,® Winthrop-Stearns, Inc., New York, N. Y., Control No. R-012-JE.

[13] Aureomycin® hydrochloride, Lederle Laboratories, American Cyanamid Company, Pearl River, N. Y., Lot No. 7-8889.

[14] Cutter Laboratories, Berkeley, Calif. Lot No. D-9743, potency approximately 1,545 units per mg.

medium has been found useful for determining the susceptibility of tubercle bacilli to streptomycin and isoniazid,[3, 4] it did not prove quite as satisfactory for testing the chromogens. This is due to the fact that these particular strains do not grow as luxuriantly on the blood medium as on the Löwenstein-Jensen medium. Hosty *et al.*[15] found a large number of chromogens that did grow as well on the blood medium prepared with blood agar base as on the Löwenstein-Jensen medium, so perhaps other chromogenic acid-fast bacilli could be tested as easily on both types of medium. Whether these differences in the degree of growth were due to the type of agar used is not known, as this factor was not tested. Another difficulty is that some of the lesser pigmented strains are not as easily seen macroscopically on the blood medium as on the egg medium, with its more contrasting background of malachite green. However, under the stereoscopic microscope, the colonies can be readily visualized. Thus, the choice of a medium for determining the susceptibility of chromogenic acid-fast bacilli to antimicrobial agents would depend partly upon how well the strains grow on the medium and how easily growth can be seen.

In summary: In the experiments described, it has been demonstrated that a number of chromogenic acid-fast bacilli isolated from human sources could be inhibited *in vitro* by several antimicrobial agents. The greatest inhibitory capacity was exhibited by streptomycin, followed in descending order by para-aminosalicylate, viomycin, oxytetracycline, chlortetracycline, and penicillin. Isoniazid failed to inhibit any of the strains in any concentration. Although several of these organisms were completely inhibited by the smallest concentrations of streptomycin or para-aminosalicylate, the over-all pattern of inhibition by the other agents when tested alone was not striking. The inhibitory effect produced by combinations of several of these agents was ve y much more pronounced than that exhibited by any single agent.

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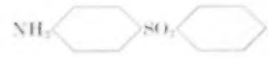
EXCRETION PRODUCTS OF 4:4′-DIAMINODIPHENYL SULFONE

The activity of 4:4′-diaminodiphenyl sulfone (Dapsone®) against streptococci was first reported in 1937 by Buttle and associates[1] and by Fourneau and co-workers[2]. The drug was considered too toxic for clinical use, however, and attempts were made to lessen the toxicity by substitution of the hydrogens of the amino groups. In 1940, Rist, Bloch, and Hamon[3] demonstrated the antituberculous activity of the parent substance and the two di-substituted derivatives, promanide (Promin®) and solapsone (sulphetrone) were used for the treatment of tuberculosis in man. Some success was claimed, but it was almost immediately overshadowed by that derived from the use of streptomycin. In the meantime, however, both 4:4′-diaminodiphenyl sulfone and its di-substituted derivatives were successfully used for the treatment of leprosy and they are still regarded as the best drugs available for the treatment of this disease.

TABLE OF FORMULAS

1. Sulfanilamide

2. 4:4′ Diaminodiphenyl sulfone

3.  Mono-anil of 4:4′-diaminodiphenyl sulfone and glucuronic acid

4. Semi solapsone

The antibacterial activity of 4:4′-diaminodiphenyl sulfone was first examined because of its close chemical relationship with sulfanilamide. Both substances have two amino groups (*see* formulas 1 and 2) but, in the sulfanilamide molecule, only one is attached directly to the benzene ring and the antibacterial activity of sulfanilamide apparently depends upon this amino group's being free. In 4:4′-diaminodiphenyl sulfone, both amino groups are attached to a benzene ring and from analogy with sulfanilamide and its derivatives it has been generally accepted that the antituberculous and antileprotic activities of the drug depend upon these groups' being free. The sulfone derivatives solapsone and promanide, which have both of their amino groups substituted, ought therefore to be inactive unless amino groups become free *in vivo*. The investigations by Titus and Bernstein,[4] Francis and Spinks,[5] and Lowe[6] leave little doubt that, when given orally, they are in fact converted to the parent sulfone in the stomach before being absorbed.

This explanation does not fully account, however, for the activities of the di-substituted forms of 4:4′-diaminodiphenyl sulfone, for, according to Cochrane[7], solapsone given

[1] BUTTLE, G. A. H., *et al.*: Lancet, 1937, *1*, 1331.
[2] FOURNEAU, E., *et al.*: Compt. rend. acad. Sc. Paris, 1937, *204*, 1763.
[3] RIST, N. F., *et al.*: Ann. Inst. Pasteur, 1940, *64*, 203.
[4] TITUS, E., AND BERNSTEIN, J.: Ann. New York Acad. Sc., 1949, *52*, 719.
[5] FRANCIS, J., AND SPINKS, A.: Brit. J. Pharmacol., 1950, *5*, 565.
[6] LOWE, J.: Leprosy Rev., 1952, *23*, 4.
[7] COCHRANE, R. F.: Practitioner, 1951, *166*, 373.

parenterally is very effective in the treatment of leprosy, and Francis and Spinks[8] found that very little of the drug was changed to 4:4′-diaminodiphenyl sulfone when given by this route. The observation by a number of workers that 4:4′-diaminodiphenyl sulfone itself undergoes a change in the body also deserves consideration when attempting to explain the *in vivo* activity of these sulfones. These workers have noted that a high proportion of the 4:4′-diaminodiphenyl sulfone excreted in the urine is in a form less readily extracted by organic solvents than is 4:4′-diaminodiphenyl sulfone. The substance is easily diazotizable, showing that at least one of the amino groups is free or potentially free, and it may be that the *in vivo* activity of 4:4′-diaminodiphenyl sulfone is due therefore to this metabolic product.

The writers have examined the excretion products of 4:4′-diaminodiphenyl sulfone and its substituted derivatives using paper chromatography and paper electrophoresis in an attempt to account for the activity of solapsone and to identify the excretion product of 4:4′-diaminodiphenyl sulfone.

Excretion products of 4:4′-diaminodiphenyl sulfone: Paper chromatography using butanol-water as the solvent shows that, in rabbits after the oral administration of 100 mg. of 4:4′-diaminodiphenylsulfone per kg., there are three substances present in the urine which can be readily detected with the *p*-amino dimethyl benzaldehyde reagent of Ehrlich. These substances, two of which are present in relatively small amounts, must therefore have amino groups which are either free or become free under the acid conditions of the test. One of the minor substances which travels fast in four different solvents is apparently 4:4′-diaminodiphenyl sulfone in unchanged form. Paper electrophoresis shows that the other minor and the major substances possess acid groupings. They are also acid-labile, decomposing to glucuronic acid and 4:4′-diaminodiphenyl sulfone when run on paper chromatograms with butanol-acetic acid. The presence of acid groupings in these components indicates that glucuronic acid is combining through its aldehyde group with 4:4′-diaminodiphenyl sulfone. Recovery of intact 4:4′-diaminodiphenol sulfone strongly suggests that the drug is being detoxicated by the formation of a Schiff's base or anil, a hitherto unrecognized method of conjugation.

The chromatographic and paper electrophoretic patterns of these excretion products of 4:4′-diaminodiphenyl sulfone in rabbit urine were reproduced exactly by a solution of glucuronic acid and 4:4′-diaminodiphenyl sulfone refluxed in acetone and water, and analysis of the components of this solution eluted from a chromatogram showed that the solution contained, besides unchanged 4:4′-diaminodiphenyl sulfone, the mono- and di-anils of 4:4′-diaminodiphenyl sulfone with glucuronic acid. The major excretion product in rabbit urine corresponds in its chromatographic behavior to the mono-anil (formula 3).

The excretion products in human urine after 300 to 400 mg. of oral 4:4′-diaminodiphenyl sulfone were at first thought to be identical with those present in the rabbit urine, an exact match in pattern being obtained on paper electrophoresis and with chromatograms run in phenol, butanol-water, and butanol-propanol-water. With butanol-acetic acid, however, the major component showed no decomposition, thus differing from the corresponding component, the mono-anil, of rabbit urine. Identical behavior in all respects except this lability to weak acid, however, makes it probable that the two substances are structurally very similar. Such similarity of structure would be possible if one of the amino groups of 4:4′-diaminodiphenyl sulfone was oxidized to a hydroxyl or carboxyl group and the resulting substance excreted as a glucuronide. Attempts to isolate the conjugate in order to characterize it have not so far been successful as the quantity excreted is so small (approximately 10 mg. per liter), but experiments to this end are continuing.

In man very little of the drug is excreted as free 4:4′-diaminodiphenyl sulfone, and ex-

traction with ethyl acetate of urine from patients on continuous oral sulfone therapy showed that less than 5 per cent of the drug is present in this form.

Excretion products of substituted 4:4′-diaminodiphenyl sulfone: Using these same methods the writers have confirmed that the di-substituted derivative of 4:4′-diaminodiphenyl sulfone, solapsone, is presumably converted to 4:4′-diaminodiphenyl sulfone because the excretion products are identical with those of the parent sulfone when given orally. We have also confirmed that solapsone given parenterally to man and rabbits is not changed to 4:4′-diaminodiphenyl sulfone, but we find that commercial solapsone contains as much as 15 per cent semi-solapsone, i.e., 4:4′-diaminodiphenyl sulfone, with the solapsone substituent on only one of the amino groups (formula 4). This mono-substituted derivative of 4:4′-diaminodiphenyl sulfone would be expected to have antibacterial activity, and this has been confirmed experimentally. It was also found that the urine of rabbits which received subcutaneous injections of solapsone free of semi-solapsone contained considerable amounts of semi-solapsone.

All the substituted derivatives are not converted in the stomach to 4:4′-diaminodiphenyl sulfone before being absorbed. Chromatography shows that the mono-substituted glycine derivative of the parent sulfone, 4-amino:4′carboxymethylamino diphenyl sulfone, was excreted unchanged after both oral and parenteral administration to the rabbit.

* * *

Although the concentration of Ehrlich-positive material in the blood after oral 4:4′-diaminodiphenyl sulfone is too low for its chemical nature to be determined directly by the methods used, there can be little doubt that 4:4′-diaminodiphenyl sulfone exists in the body in rabbit and man almost entirely in combination with glucuronic acid, with one NH_2 group remaining free. It is therefore tempting to assume that the antibacterial activity of 4:4′-diaminodiphenyl sulfone in man is due to this mono-substituted derivative and that the presence of two free amino groups are not necessary for antituberculous and antileprotic activities. That mono-substituted 4:4′-diaminodiphenyl sulfone derivatives have antituberculous activity in guinea pigs was shown by Smith, Jackson, and Bauer[6]. In fact, they found some to be more effective than the parent substance. Also, Cochrane[7] has shown that the mono-substituted glycine derivative, which the writers have shown is excreted unchanged, is effective in the treatment of leprosy. Hence, the activity of parenteral solapsone can be accounted for both by the presence of semi-solapsone in the commercial drug and its formation *in vivo*.

If it is true that the *in vivo* activity of 4:4′-diaminodiphenyl sulfone is due to a mono-substituted derivative, which the body has produced in an effort to detoxicate the drug, it would not seem reasonable to administer the drug in an unsubstituted form. All mono-substituted derivatives are not of equal antituberculous activity and there is no reason to expect the body to produce, in its effort to detoxicate 4:4′-diaminodiphenyl sulfone, the most effective antileprotic derivative. In fact the experiments of Smith and associates[6] suggest that more effective antileprotic mono-substituted derivatives can be prepared by varying the substituent.

Details of the experiments discussed in this preliminary communication will be described elsewhere.

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January 31, 1955

[6] SMITH, M. I., *et al.*: Ann. New York Acad. Sc., 1949, *52*, 704.

INSTABILITY OF THE POTENCY OF TUBERCULIN DILUTIONS

During the course of field studies on the stability of dilutions of purified tuberculin (RT XIX-XX-XXI), considerable variations in potency were found not only from dilution to dilution but even from bottle to bottle filled with the same dilution. As the variations could not be ascribed to the duration or temperature of storage, or to any other factor known to the writers, a series of studies were begun in an attempt to find, and eliminate, the source of variability. Among these studies, certain findings are so striking as to warrant a preliminary report at this time.

Adsorption of protein to the glass bottles was considered a possible cause, as the degree of such adsorption might well be expected to vary from bottle to bottle. Attempts were therefore made to saturate the glass surface by filling bottles with tuberculin dilution and allowing them to stand overnight before emptying and refilling them with fresh dilution. The contents were then compared with a portion of the same dilution in "unsaturated" bottles by testing groups of BCG-vaccinated school children. The results sometimes, but not always, showed a difference in potency between the dilutions in "unsaturated" and "saturated" bottles.

Another way of investigating the "adsorption theory" was to vary the volume of tuberculin in the bottles, assuming that the larger the ratio of glass surface to volume, the greater would be the adsorption effect. Surprisingly large differences were found in the potency of tuberculin from partially filled bottles as compared with completely filled bottles, as shown by the results of four studies given in tables 1 through 4.

In table 1 the results are summarized of a study carried out in a total of 487 BCG-vaccinated school children, each given a test with 5 tuberculin units (TU) (0.0001 mg. in 0.1 ml.). Half of the children were tested with dilutions from completely filled 20 ml. bottles; the other half, with a portion of the same dilution from bottles filled with just 2 ml. (In this and the following studies, less than 1 ml. of dilution was used from each bottle.) Tuberculin from bottles filled with 20 ml. gave, on the average, reactions 7.2 mm. larger than the same tuberculin from the same kind of bottles containing only 2 ml. The difference, which statistically is highly significant, is of the same order of magnitude as the difference between a 5 TU and a 1 TU dilution.

The study was repeated two weeks later in another group of 305 school children and, as shown in table 2, the "volume effect" was again pronounced. In this study, the tuberculin dilution had been prepared and the bottles filled with 20 ml. and 2 ml. portions approximately twenty-four hours before use.

The results of these two studies, while showing essentially the same "volume effect," raised the question whether or not, in everyday tuberculin testing work, the tuberculin used for tests given out of an almost empty bottle would be substantially weaker than that used for tests given when the bottle was full. Accordingly, the next study was designed to approach the question experimentally. To avoid possible difficulties with the local health authorities because of the surprisingly "poor" tuberculin reactions in the BCG-vaccinated children, 7 ml. were used instead of 2 ml. for the partially filled bottles in this study.

Tuberculin from completely filled 20 ml. bottles was compared with portions of the same dilution from bottles filled with only 7 ml. in three different ways: in some, 13 ml. were withdrawn from completely filled 20 ml. bottles a few days before use; in others, 13 ml. were withdrawn a few hours before use; and, in the rest, the bottles were originally filled with only 7 ml. A total of 315 BCG-vaccinated school children were tested and,

TABLE 1

MEAN SIZE OF INDURATION OF REACTIONS TO 5 TU AMONG 487 BCG-VACCINATED SCHOOL CHILDREN ACCORDING TO THE VOLUME OF THE TUBERCULIN DILUTION IN 20 ML. BOTTLES

Volume of Dilution in 20 Ml. Bottle	Mean Induration	Number Tested
	mm.	
20 ml.	17.1	243
2 ml.	9.9	244

TABLE 2

MEAN SIZE OF INDURATION OF REACTIONS TO 5 TU AMONG 305 BCG-VACCINATED SCHOOL CHILDREN ACCORDING TO THE VOLUME OF THE TUBERCULIN DILUTION IN 20 ML. BOTTLES

Volume of Dilution in 20 Ml. Bottle	Mean Induration	Number Tested
	mm.	
20 ml.	14.5	151
2 ml.	8.3	154

TABLE 3

MEAN SIZE OF INDURATION OF REACTIONS TO 5 TU AMONG 315 BCG-VACCINATED SCHOOL CHILDREN ACCORDING TO THE VOLUME OF THE TUBERCULIN DILUTION IN THE 20 ML. BOTTLES BEFORE USE

Volume of Dilution in 20 Ml. Bottle	Filling Procedure	Mean Induration	Number Tested
		mm.	
20 ml.	Bottles filled with 20 ml., none withdrawn	17.6	80
7 ml.	Bottles filled with 20 ml., 13 ml. withdrawn 5 days before use	15.8	82
7 ml.	Bottles filled with 20 ml., 13 ml. withdrawn a few hours before use	15.6	76
7 ml.	Bottles filled with 7 ml., none withdrawn	15.2	77

TABLE 4

MEAN SIZE OF INDURATION OF REACTIONS TO 5 AND 10 TU AMONG BCG-VACCINATED GUINEA PIGS ACCORDING TO VOLUME OF TUBERCULIN DILUTION IN 20 ML. BOTTLES

Volume of Dilution in 20 Ml. Bottle	5 TU		10 TU	
	Mean Induration	Number of Tests	Mean Induration	Number of Tests
	mm.		mm.	
20.0 ml.	9.3	12	11.0	12
7.0 ml.	8.0	12	10.2	12
2.5 ml.	4.6	12	5.8	12

as shown in table 3, the contents of the three groups of bottles containing 7 ml. were less potent than the contents of the completely filled bottles. These results suggest that the potency of tuberculin is reduced when the volume in the bottle is reduced, regardless of how the reduction in volume is brought about.

As further study of the problem would require greater experimental freedom than could be accepted in human beings, the experimentation was continued in guinea pigs. In table 4 the results of one of the small-scale preliminary experiments are shown. Twenty-milliliter bottles were filled with 20 ml., 7 ml., and 2.5 ml. of 5 and 10 TU dilutions, and tests were given with tuberculin from each of the six bottles, twelve tests from each. Despite the small number of tests, the results showed the same clear pattern which was found in the studies on school children.

These studies were done with a purified tuberculin product RT XIX-XX-XXI. Very recent experiments not reported here indicate, however, that such results are not reproduced with another tuberculin product.

The results given in this preliminary note have been selected from a series of studies to illustrate that the potency of tuberculin may be affected, often drastically, by factors as yet unrecognized. In the hope of eventually being able to identify the causal factors, further studies are now being planned along different lines of approach, including physico-chemical experimentation, trials with various tuberculin preparations, et cetera.

World Health Organization Tuberculosis Research Office — Johannes Guld, Knut Magnus
and
Statens Seruminstitut, Copenhagen, Denmark — Mogens Magnusson

March 18, 1955

Correspondence

BERYLLIUM CASE REGISTRY AT MASSACHUSETTS GENERAL HOSPITAL

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

Your readers may be interested in the first progress report of the beryllium case registry being established at the Massachusetts General Hospital in Boston.

At this time, 271 cases reasonably well documented are available for study. It is planned to handle this material anonymously in order to preserve the privacy of physician-patient relationship and make it possible to open the registry for study to any interested individual. The data is being put onto Keysort cards, the individual patient being identified by an assigned number. We plan to get evidence that can be used not only to describe beryllium disease more exactly but to increase knowledge of its epidemiology, the value of our present methods of control of hazardous operations and, finally, of the usefulness of present forms of treatment. By late 1952 we had gradually collected 90-odd cases about which we had enough information to justify including them in the so-called registry. By June, 1954, there were 271 cases answering this description. This report was prepared chiefly by Dr. Warren Weil, who worked full time on this project from December, 1952, to June, 1954. Dr. Weil points out that the 271 cases do not include the "significantly larger group of leads, prospects, incompletes, questionables, and new cases" that remain to be studied.

Clinical classification: The group has been further broken down into four groups of general classification: (*1*) The acute group includes those patients whose onset of illness followed soon after or while still being exposed. (*2*) The chronic group includes those cases with onset often after years out of exposure with clinical picture of chronic beryllium disease. (*3*) The acute to chronic group is, as the title implies, a small number of patients whose history indicates either gradual, or abrupt after considerable delay, transition from the primary pulmonic manifestations of the chronic form. (*4*) The exposed-asymptomatic group are patients with a history of exposure to beryllium sufficient to cause illness in others, with roentgenographic evidence of alteration in the normal parenchymal pattern consistent with changes secondary to beryllium, who are without symptoms. Patients in this latter group may also have a urinalysis positive for beryllium.

1. Acute—63
2. Chronic—207
3. Acute to chronic—6
4. Exposed-asymptomatic—14

Total—290

(The total of 290 is greater than the case total because cases of acute to chronic are included in both groups, acute and chronic. Also, cases in the asymptomatic group may also appear in the group of acute or chronic cases, to which they once belonged.)

Classification according to industry, with present available information, results in four sub-groups: (*1*) fluorescent (referring to those patients exposed to beryllium-containing phosphors), (*2*) extraction (describing those who were employed by firms extracting the metal from beryl ore), (*3*) other (referring to those handling beryllium or its compounds in a variety of operations later to be completely identified), and (*4*) nonoccupational (describing those patients who were not employed in the manufacture or use of beryllium

but who lived or worked in proximity to atmospheric contamination with beryllium). These include acute and chronic.

1. Fluorescent—126 (or 46% of total)
   Acute 2 Chronic 124
2. Extraction—75 (28% of total)
   Acute 50 Chronic 25
3. Other—44 (16% of total)
   Acute 4 Chronic 40
4. Nonoccupational—26 (10% of total)
   Acute 0 Chronic 26

Examination of these figures points to the possibility of one or multiple decisive factors. Intensity of exposure, characteristics of the beryllium compounds involved, and/or association with other potentially harmful materials may account for the predominance of the acute disease in the extraction group and the consistent predominance of the chronic form in the other categories. Much knowledge of exposed population will be needed to interpret the "non-occupational" group.

Mortality: Present information on mortality:

Males living 124 (or 78%)	Females living 75 (or 68%)
Males dead 36 (or 22%)	Females dead 36 (or 32%)
160	111

Deaths—72:

Fluorescent—38
Acute 1 Chronic 37
Autopsy—26

Extraction—14
Acute 11 Chronic 3.
Autopsy—11

Other—10
Acute 1 Chronic 9
Autopsy—9

Non-Occupational—10
Acute 0 Chronic 10
Autopsy—7

These figures illustrate the significant mortality associated with illness, acute or chronic, that arises from contact with certain beryllium compounds. We do not have as yet the complete record of the dates of death of these patients. It is possible that study of these would indicate a fairly consistent limit to the duration of the fatal cases of acute disease (none more than sixty days from onset to demise) and a definite trend to an increasing span of life from onset to demise in the cases of the chronic disease, with a steady decline in the number of deaths per year after the initial flurry of fatalities in the mid-forties. Of 72 deaths, 53 autopsies are known to have been done.

Although the exact dates of death are not in our data, it is interesting to note that, of the 53 autopsied patients, 17 per cent of the deaths were in the five-year period prior to 1945; 72 per cent of the deaths were between 1945 and 1949; and 11 per cent have occurred from 1950 to date. The autopsy appears valuable in confirming the clinical impression. In the 53 autopsies we have knowledge of the findings regarding the lungs in 45 cases (85 per cent). The lung was diagnosed as showing pathology consistent with beryllium disease in every case (100 per cent). Spectrographic analysis was performed on more than half of these patients (exact figures not yet available) and was reported as showing no beryllium only twice. The history of exposure and the morphologic lesions were strongly confirmatory of a positive diagnosis in both of these latter cases.

Evidence to support the impression that the chronic form of the disease is systemic

is found in the autopsy data. In addition to finding beryllium in the lungs in each of the 45 cases known to us, there were liver lesions in 13 cases (confirmed by finding beryllium on analysis), kidney lesions in 6 cases, splenic lesions in 12 cases, lymph node lesions in 8 cases, and lesions were also noted, in a single case, in cartilage and adrenal glands. Spectrographic analysis of bone was positive in 4 of these cases, and 5 cases proved by assay to have beryllium in the myocardium. Carcinoma complicated 2 cases. One patient with a history of the acute disease died with carcinoma of the lung. One other patient was known to have a carcinoma of the prostate at the time of his death (no autopsy in this latter case). Four patients had tuberculosis as an associated finding, confirmed at autopsy in 2 cases. Two of the patients coming to autopsy with the chronic form of the disease died soon after a large spontaneous pneumothorax.

The value of biopsy in the diagnosis of this disease is illustrated by the fact that the pathologic diagnosis was consistent with beryllium disease in all 19 (14 males, 5 females) of this group subjected to lung biopsy. Seventeen of these showed detectable amounts of beryllium on spectrographic analysis. Liver biopsy has not been so helpful, with only one positive diagnosis in 5 attempts on 4 patients. Biopsy of lymph nodes has been positive in 2 of 3 patients, and skin biopsy has shown lesions consistent with beryllium in 2 of 3 patients in this group of 271. A single biopsy of the tibia was negative on analysis. Beryllium was found in rib sections removed during thoracotomy for lung biopsy in 2 of 5 patients.

Spectrographic analysis of body fluids has been carried out in many of these cases.

Urine—61 (38, or 62% positive)
Acute—16 (14, or 88% positive)
Chronic—45 (24, or 53% positive)
Blood—21 (3, or 14% positive)
Acute—8 (1, or 13% positive)
Chronic—13 (2, or 15% positive)

In 15 cases both urine and blood were analyzed from the same patient; in no case were both positive. In 10 cases, the urine was positive in the presence of a negative blood. In 5 cases both were negative. In no case was the blood positive in the presence of a negative urine.

Kidney calculi, a complication of beryllium disease, were analyzed for beryllium in 2 cases and on both occasions were positive.

Pulmonary function studies have been done on 27 of these patients (24 males, 3 females) and in no instance in which there was evidence of beryllium disease was there normal function.

Present knowledge of therapy with steroids: Eighty-six of this group received steroid therapy, cortisone, corticotropin, or both, with a remarkably low incidence of the common complications. There were 2 episodes of transient psychotic behavior during the use of corticotropin. During cortisone treatment, there were 2 instances of transient glycosuria, 2 instances of symptoms of peptic ulcer, with negative roentgenographic studies, controlled by a medical regimen, and 2 instances of fractured ribs. In only one case was the drug discontinued because of these complications.

Although we are not able to present statistically controlled data at this time, it is worth including the strong impression from the material at hand that the use of cortisone, with or without an occasional course of intramuscular corticotropin, is of real benefit in the relief of the symptoms of beryllium disease. Lung function studies following cortisone therapy demonstrate that the pathology resulting in faulty diffusion of gases has definitely

been altered. The average steady dose of cortisone in cases known to us is 75 mg. a day. It is hoped, when this report is presented a year from now, that controlled figures can be given.

The beryllium case registry is completely dependent upon information that we can get through the cooperation of plant physicians, family doctors, and pathologists who may have had a chance to study autopsy or biopsy material. Through the help we receive from the Atomic Energy Commission, Division of Biology and Medicine, it is possible to offer diagnostic help or analysis of tissue or body fluids to physicians who are trying to make a diagnosis of beryllium disease. Please feel free to correspond with us by addressing The Beryllium Case Registry, Massachusetts General Hospital, Box 703, Boston, Massachusetts.

Massachusetts General Hospital
Boston 14, Massachusetts

Sincerely yours,
Harriet L. Hardy

March 16, 1955

Books

MEDICAL SCHOOLS IN THE UNITED STATES AT MID-CENTURY. By John E. Deitrick and Robert C. Berson. McGraw-Hill Book Company, Inc., New York, Toronto, and London, 1953.

Without further ado it must be stated that this is a book that should be owned by all teachers of medicine from intern to professor. Here, we have the problems, the aspirations, and the achievements of American medicine. Medicine is a synthesis and application of science than which there is no greater. The facts are recorded here by two careful observers, Dr. Deitrick and Dr. Berson. These facts are culled from reports of a committee dealing with a survey of the last ten years of education.

The recurrent theme of this book is "What is medical education?" It can be none other than the education of medical students to develop their capabilities maximally, to obtain a firm grasp of the experimental method, and to study diseased patients sympathetically. Many forces are at work diverting educators from this basic aim. Some medical schools are engaging in "health personnel education" (nurses, dentists, technicians et cetera) to the detriment of the education of the medical student. Other schools are undertaking extensive medical "service"—to the communities, hospitals, et cetera—under the guise of medical education. Emphasis on research causes it to be forgotten that teaching is vitalizing, too, and that the young inquiring mind of students is something that is missing from research institutes.

The book is organized in five divisions: functions, finances, operations, curriculum, and teaching methods and the advanced education and training of medical schools. Each chapter is followed by "Conclusions" and "Recommendations" for easy revision. All activities except research are less well financed in 1950 than in 1941. In some respects this is a result of poor cost accounting, so that projects of research do not contribute enough to general upkeep. For the same reason the "cost" of educating a medical student has been given a very unreal figure. Of the 8.5 billions spent by the American public on medical services, only 106 millions is spent on the medical schools—they can well afford to spend more on the main factor determining the future of medicine in the United States.

Forces within the medical school leading to recent change are the great growth of chemistry in relation to medicine, help asked by society in the social fields, increased nonmedical students in the basic sciences causing organization around subject matters rather than students, the great development of clinical departments, and the requirements of specialty boards.

While the need for full-time men is clear, their choice in most cases is for qualities other than teaching. The tremendous increase in the duties of a dean are dealt with at length. It is pointed out that deans should be directly responsible to presidents of universities, and university hospital directors should be responsible to the deans.

While private grants have quadrupled in ten years, a trend to tax support is unmistakable. The drain of medical school funds through the care of charity patients and other hospital functions should stop.

An interesting prediction merits quotation in full:

> Training for the type of general practice that may be envisioned for the future should be such as is now given to the internist, including pediatrics and psychiatry and perhaps also some obstetrics and minor surgery. The training must be thoughtfully designed to develop competence in diagnosis and real skill in treatment.

Fundamentally, emphasis must be placed on quality of instruction, and the vision of greatness must be preserved. This book points the way to this goal in a clear and critical fashion.

J. MacLean Smith
New York, New York

Sandoz Atlas of Haematology. Pp. 91, with illustrations, Sandoz Blood Atlas, Route 10, Nutley, New Jersey.

The revised *Sandoz Atlas of Haematology* is now available in English as well as in French, German, and Italian editions. Produced in the form of a large looseleaf notebook, the volume contains a fairly detailed description of the various normal and abnormal blood and marrow elements, with a review of histogenesis and classification and a brief description of staining techniques. The text on morphologic hematology reflects the European origin of the book, which was prepared in the Sandoz Pharmacological Research Laboratories in Basle. Some of the authors' terms and concepts will not appeal to American hematologists.

There are fifty full pages of magnificent reproductions of photomicrographs of blood and marrow cells and blood parasites. These illustrations, all in full color and at high magnification, are remarkably beautiful and accurate and vastly superior to those found in several other atlases currently available. Illustrations of Gaucher's cells are disappointing in that they do not show the fibrillar appearance of the cytoplasm so characteristic of these cells. Figures representing the "L. E." cells of lupus erythematosis provide very poor examples of these structures. However, most of the reproductions are extremely good and are a tribute to the skill and artistry of the photographer and printer. The volume should be greatly valued wherever an atlas of hematology is employed.

C. Lockhard Conley
Baltimore, Md.

Ergebnisse der Gesamten Tuberkuloseforschung. Volume 12. Edited by H. Beitzke, St. Engel, L. Heilmeyer, J. Klein, and E. Uehlinger. Pp. 488, with illustrations, Georg Thieme Verlag, Stuttgart, 1954.

The late professor Beitzke has written one of the chapters in this volume and actively edited it until he died in June, 1953.

The first 42 pages contain articles by Engel, Beitzke, and Dufort and Depierre concerning bronchoperforation of tuberculous lymph nodes, which continues to be a problem of considerable interest. The next 100 pages, written by Wurme, comprise a good survey on atelectasis in tuberculosis. The article on asthma and pulmonary tuberculosis is written by Mai.

Endocrinology and tuberculosis, by Schaefer, is a 120-page section containing current knowledge on this very interesting problem. The 25-page bibliography will be of great help to those interested in this subject.

There is a clinical description of fourteen cases of tuberculosis of the spleen by Wetzel. Spiess gives a good survey of the literature on experimental BCG studies. The practical, as well as theoretical, problems concerning the effect of superinfection on the course of tuberculosis in children, are reviewed by Vogt in an objective way.

As the editors state in the preface, although the new *Ergebnisse* contains subjects of timely interest, chemotherapeutic problems will not be presented until the next volume of this series.

It will not be attempted to review in detail all of the interesting points brought out in this 488-page volume. It must be emphasized, however, that the subjects treated in this book, by some very well-known European authors, are of sufficient interest to recommend a cover-to-cover reading.

This edition is excellently published, printed on good paper, with instructive and clear pictures. The systematic way of presenting the articles is much like that of a monograph. The extensiveness of the literature references and the discussions in many of these review articles, expressing the authors' critical viewpoint, make the volume of great value. There is also a good author and subject index.

The twelfth volume of *Ergebnisse der Gesamten Tuberkuloseforschung* is to be recommended to phthisiologists, pediatricians, and especially to investigators interested in subjects dealt with in this volume.

Ole Wasz-Höckert
New York, New York
(Helsinki)

Unsere Erfahrungen über die Moderne Behandlung der Miliartuberkulose und der Meningitis Tuberculosa im Kindesalter. By J. R. Weber and K. Kumin. Pp. 95, Verlag Hans Huber, Bern and Stuttgart, 1954.

The case histories of forty-two patients treated for miliary and/or meningeal tuberculosis during the period 1949 through 1953 are reported.

Twenty-nine of the forty-two patients received streptomycin—PAS, whereas the other thirteen were treated with isoniazid—streptomycin—PAS. The thirty-seven patients with meningitis were treated with streptomycin administered intrathecally in addition to the above regimens.

There were nine deaths (a 21.4 per cent mortality) in the total group of forty-two patients. Excluding the patients with only miliary disease, the mortality of the meningitis group was 32.1 per cent. The group of patients who received isoniazid had a clearly demonstrably lower death rate. There were no complications in twenty-two of the thirty-three survivors.

The prognosis was considerably better in the older age groups. It was particularly poor in the patients two or three years of age.

A short but well-selected literature survey is given. Summaries in English, French, and Italian are available.

The book is recommended to all who are interested in the management and chemotherapy of miliary-meningeal tuberculosis. Pediatricians especially will find this book valuable.

Ole Wasz-Höckert
New York, New York
(Helsinki)

BOOKS RECEIVED

Fluoroscopy in Diagnostic Roentgenology. By Otto Deutschberger. Pp. 771, with illustrations, W. B. Saunders Company, Philadelphia and London, 1955, Price, $22.00.

Segmental Anatomy of the Lungs. By Edward A. Boyden. Pp. 276, McGraw-Hill Book Company, Inc., New York, 1955, Price, $15.00.

The Lung. Clinical Physiology and Pulmonary Function Tests. By Julius H. Comroe,

Jr., Robert E. Forster, Arthur B. Dubois, William A. Briscoe, and Elizabeth Carlsen. Pp. 219, The Year Book Publishers, Inc. Chicago, 1955, Price, $5.50.

Heures Internationales dans la Lutte Contre la Tuberculosis. By Étienne Bernard. Pp. 89, Masson et Cie, Paris, 1955.

La Tuberculose Primaire Chez L'Enfant. By Raymonde Grumbach, in collaboration with Sonia Gerbeaux. Pp. 92, Expansion Scientifique Française, Paris, 1955.

Bronchus und Tuberkulose. By A. Huzly and F. Böhm. Pp. 138, with illustrations, Georg Thieme Verlag, Stuttgart, 1955, Price, $13.55.

Medical Progress—1955. A Review of Medical Advances During 1954. Edited by Morris Fishbein. Pp. 346, McGraw-Hill Book Company, Inc., New York, 1955, Price, $5.00.

Tuberculosis. Classification, Pathogenesis and Management. By Milosh Sekulich. Edited by H. Stanley Banks. Pp. 316, William Heinemann Medical Books, Ltd., London, 1955.

Pulmonary Diseases. Edited by Roscoe L. Pullen. Pp. 669, with illustrations, Lea & Febiger, Philadelphia, 1955, Price, $15.00.

Antibiotics Annual. 1954–1955. Edited by Henry Welch and Félix Marti-Ibanëz. Pp. 1154, Medical Encyclopedia, Inc., New York, 1955, Price, $10.00.

American Trudeau Society

Medical Section of the National Tuberculosis Association

Report of Eleventh Pembine Conference

The Eleventh Pembine Therapy Conference met at the Four Seasons Club, Pembine, Wisconsin, on September 10, 11 and 12, 1954. Fifty conferees were from the three states of Minnesota, Michigan, and Wisconsin. The ten guests were:

John B. Barnwell—Washington, D. C.
Martin M. Cummings—Washington, D. C.
Lawrence H. Kingsbury—Orlando, Fla.
Esmond R. Long—Philadelphia, Pa.
William L. Potts—Lantana, Fla.
Marjorie Pyle—Chicago, Ill.
Edward L. Ross—Winnipeg, Man.
Ivan C. Schmidt—Lantana, Fla.
Julius L. Wilson—Philadelphia, Pa.
Robert L. Yeager—Pomona, New York

Drs. Potts and Schmidt were the guest presenters.

The consecutive case presentations this year consisted of 30 consecutive patients recommended for excisional surgery or collapse procedures on and after July 1, 1953. Presentations of case series were made by four hospitals:

Minnesota—Ancker Hospital, St. Paul
Michigan—Michigan State Sanatorium, Howell
Wisconsin—Veterans Hospital, Madison
Florida—Southeast Florida Tuberculosis Hospital, Lantana

The stage of disease in each series was:

	Minnesota	Michigan	Wisconsin	Florida
Minimal	2	1	2	0
Moderately advanced	20	15	10	7
Far advanced	8	14	18	23

The shift among the Florida patients was attributed to the high Negro population in the hospital. Some differences were noted between hospitals in the types of patients.

Resectional surgery was recommended almost exclusively in the cases presented by Minnesota, Wisconsin, and Florida. Temporary collapse procedures predominated in the Michigan series. All patients were receiving chemotherapy. Multiple resectional procedures were used in all series, both unilateral and bilateral, so that a tabulation of these is not possible. Single and multiple segmental resections were the procedures of choice in the majority of cases. Surgery recommended was refused by only 6 patients of the total of 120. Two operative deaths occurred among the four series, involving 163 operative procedures.

The cases presented gave an excellent opportunity to evaluate the surgical and collapse measures employed most commonly at the present time. Resectional surgery was given the most critical appraisal observed at a Pembine Conference. The trend toward more conservative criteria was more definite than last year.

There was very little support for the use of temporary collapse measures, such as phrenic nerve operations and pneumoperitoneum. It was believed that comparable results could be obtained with chemotherapy alone, that in most patients who were receiving temporary collapse resection was indicated, and that the use of temporary measures delayed the consideration of resectional surgery.

Resectional surgery was accepted generally as the procedure of choice when there was a persistent cavity and when there was evidence of material lung tissue destruction. There was no agreement in regard to bronchiectasis. Use of contrast media will reveal unsuspected bronchiectasis in a sizable number of cases. Its presence may influence the decisions for and the extent of resectional surgery. However, routine bronchograms were questioned because of reactions and possible obscuring of residual lesions. Tomography was considered essential, but it was believed it could not differentiate accurately the presence or absence of cavities in all cases. In reviewing cases for irreversible surgical procedures, it was agreed that the earliest films available, the films showing the maximum extent, and intermediate progress films should all be considered.

The general consensus was that small nodules could be treated adequately with chemotherapy alone. It was agreed that the exact status of these could not be determined from roentgenographic films. More information is needed about the fate of these small areas of infection but the trend is toward chemotherapy alone.

Several cases of decortication were in the series. It was emphasized that this procedure should be used only to obliterate pleural space, rather than to improve function. Minimal functional improvement can be anticipated, since changes in the lung tissue are irreversible.

Postoperative pain seemed to be unrelated to the surgical approach. The incidence of postoperative pain was the same whether a rib was or was not resected.

A plea was made for more extensive and intensive examinations of resected lung tissue. Isoniazid has altered very definitely the pathology of tuberculous disease. More detailed information is needed concerning the bacteriologic status of lesions of varying sizes. There is much uncertainty about the viability of tubercle bacilli in these lesions. The lack of growth on culture media or the inability to produce disease in the guinea pig is not a criteria of death. Guinea pigs should be inoculated, not only with material from lung lesions, but also with positive cultures of the same material. More attention should be given to improving the bacteriological techniques used. Refinements in techniques may change the results now obtained.

One session of the Conference was devoted to a symposium on bone and joint tuberculosis. The cases were presented by Glen Lake Sanatorium, Oak Terrace, Minnesota. These cases were selected to demonstrate involvement of specific joints and complex or complicating disease. Discussion of these cases was led by a panel of orthopedic surgeons:

Carl E. Badgley—Ann Arbor, Michigan
Edward T. Evans—Minneapolis, Minnesota
C. Leslie Mitchell—Detroit, Michigan
A. C. Schmidt—Milwaukee, Wisconsin

In this symposium, the clinical data was given and the hospital course presented up to a point. The discussion then centered about the therapy that seemed indicated. Following this, the measures used and the subsequent course were reviewed.

Extrapulmonary tuberculosis has been a serious disease, with a high mortality and high relapse rate. Multiple foci tend to occur in these cases. Therapy was quite prolonged and the results were uncertain. Data presented showed that the mortality rate in a baseline study of pre-chemotherapy cases was 46 per cent. Introduction of chemotherapy has re-

duced the treatment time and the mortality, but not proportionally to that observed in pulmonary disease.

Two major points were derived from the symposium. Chemotherapy has changed materially the surgical procedures and surgical therapies that can be used. There is considerable divergence of opinions as to the methods of choice. It is hoped that other symposia of this type will result in closer agreement on therapies.

Chemotherapy now permits the surgeon to treat nonpulmonary tuberculous abscesses much as pyogenic abscesses are treated. The cases presented showed this approach. Abscesses in this series were treated successfully by excision, debridement, packing, and early closure. Some early and localized bone lesions can be excised without concurrent fusion and without loss of the joint motion or function. Fusion of a joint is not required as frequently as before chemotherapy.

It was apparent to the conferees that a critical evaluation of the surgical therapy of bone and joint tuberculosis is needed. The number and types of these cases seen in most centers and clinics is small. If cases of a similar nature were collected and subjected to a critical review, the present confusion concerning the proper therapies could be reduced materially.

The next Pembine Conference was scheduled for September 9, 10 and 11, 1955.

Joseph L. Egle, Michigan
Elvind P. Fenger, Minnesota
Morris C. Thomas, Wisconsin
Chairman, Pembine Conference

NOTICES

PORTUGUESE TRANSLATION OF DIAGNOSTIC STANDARDS AND CLASSIFICATION OF TUBERCULOSIS

An excellent translation of the 1950 edition of *Diagnostic Standards and Classification of Tuberculosis* is now available. Dr. Penildon Silva, of Sao Paulo, Brazil, has translated this widely used publication and copies are now available by writing to Professor Dr. Jose Silveira, Instituto Brasileiro Para Investigacao Da Tuberculose, Caixa Postal 635, Bahia, Brasil. The price is 50 cents.

LABORATORY REFRESHER TRAINING COURSES

The following Laboratory Refresher Training Courses will be offered by the Communicable Disease Center, Chamblee, Georgia, during 1955–1956.

Laboratory Diagnosis of Bacterial Diseases
- Part 1. General Bacteriology—8.40. September 12–23, 1955
- Part 2. General Bacteriology—8.41. September 26 to October 7, 1955
- Enteric Bacteriology—8.50. October 17–28, 1955

Laboratory Diagnosis of Parasitic Diseases
- Part 1. Intestinal Parasites—8.00. September 12 to October 7, 1955
- Part 2. Blood Parasites—8.01. October 10–28, 1955

Laboratory Diagnosis of Viral and Rickettsial Diseases—8.20
October 17–28, 1955, and March 12–23, 1956

Laboratory Diagnosis of Rabies—8.26
October 31 to November 4, 1955, and March 26–30, 1956

Laboratory Methods in Medical Mycology
- Part 1. Cutaneous Pathogenic Fungi—8.15. October 31 to November 11, 1955
- Part 2. Subcutaneous and Systemic Fungi[1]—8.16. November 14–25, 1955

Laboratory Diagnosis of Tuberculosis—8.55. November 14–25, 1955

Laboratory Methods in the Study of Pulmonary Mycoses—8.17. November 28–December 9, 1955.

Laboratory Diagnostic Methods in Veterinary Mycology—9.40. December 12–16, 1955.

Laboratory Diagnosis of Malaria[2]—8.05

Virus Isolation and Identification Techniques[2]—8.21

Typing of *Corynebacterium diphtheriae*[2]—8.42

Special Problems in Enteric Bacteriology[2]—8.51

Phage Typing of *Salmonella typhosa*[2]—8.52

POSTGRADUATE COURSE IN PEDIATRIC ALLERGY

New York Medical College, Flower and Fifth Avenue Hospitals, Division of Graduate Studies, Department of Graduate Pediatrics, announces a postgraduate course in pediatric allergy under the direction of Dr. Bret Ratner, Professor of Clinical Pediatrics and Associate Professor on Immunology. The course will be held for thirty sessions, Wednesdays, 9 a.m. to 4 p.m., from November 2, 1955 to May 31, 1956. The fee is $300.

The course consists of lecture-seminars, laboratory and clinical procedures, clinic work,

[1] Completion of Course 8.15 or equivalent education or experience is a prerequisite.
[2] Courses offered by special arrangement only.

ward rounds and animal experimentation covering basic principles of diagnosis and treatment of allergy in children, and applied immunology.

Applicants must be certified in pediatrics or have the requirements for certification. Enrollment is limited.

Apply to the Office of the Dean, New York Medical College, Fifth Avenue and 106 Street, New York 29, New York.

A Research Fellowship in Pediatric Allergy is now available. Application should be made now.

POSTGRADUATE COURSE IN TUBERCULOSIS OF CHILDHOOD

The Department of Pediatrics of New York University College of Medicine is offering a course in tuberculosis for pediatricians and general practitioners. This will be given by the Chest Clinic of the Children's Medical Service of Bellevue Hospital, five days, full time, September 26–30, 1955.

This course is designed for pediatricians or general practitioners interested in tuberculosis in infants and children. It will comprise lectures, clinical demonstrations, and discussions of the diagnosis, prognosis, and treatment of tuberculosis and other pulmonary disorders. The tuition is $60.00.

Those interested should apply to Dr. Edith M. Lincoln, Bellevue Hospital, New York 16, New York.

AMERICAN HEART ASSOCIATION ANNUAL MEETING AND RESEARCH SUPPORT

The 31st Annual Meeting and the 28th Scientific Sessions of the American Heart Association will be held at the Jung Hotel in New Orleans from October 22 through October 26. The 1955 gathering will be the first held independently of any major medical meeting.

The Scientific Sessions will occupy three days, Saturday, Sunday, and Monday, October 22–24.

Applications by scientific investigators for support of research to be undertaken during the year beginning July 1, 1956, are now being accepted by the American Heart Association.

The application deadline for research fellowships and established investigatorships is September 15, 1955. Applications for grants-in-aid must be made by November 1, 1955, *a month earlier than in previous years.*

Funds to support Heart Association research projects in the cardiovascular field are provided from public contributions to the Heart Fund.

Established Investigatorships: Awarded for periods of up to five years, subject to annual review, in amounts ranging from $6,000 to $9,000, to scientists of proved ability who have developed in their research careers to the point where they are independent investigators.

Research Fellowships: Awarded to young men and women with doctoral degrees for periods of one to two years to enable them to train as investigators under experienced supervision. Annual stipends range from $3,500 to $5,600.

Grants-in-Aid: Made to experienced investigators to provide support for specific projects. Grants are made in varying amounts, not to exceed $10,000.

The American Heart Association also maintains another and unique form of research support, the Career Investigatorships. This is given to a limited number of investigative scientists of unusual capacity and accomplishment to assure them of financial support throughout their productive lives. Career investigators are selected on the initiative of the Research Committee, rather than by application.

Further information and application blanks may be obtained from The Medical Director, American Heart Association, 44 East 23 Street, New York 10, N. Y.

ERRATUM

In the Abstract Section of Volume 71, No. 5, 1955, on page 157, "Experiences in Immunization Against Tuberculosis with BCG Vaccine in Japan," the first sentence should be changed to read as follows: Between 1945 and 1952, approximately *30,000,000* people in Japan under thirty years of age. . ." instead of "approximately *30,000* people."

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Sensitivities to the commonly employed therapeutic agents are accurately and readily determined using Bacto-Sensitivity Disks containing three concentrations of Dihydrostreptomycin, Streptomycin, Viomycin, PAS (p-Amino Salicylic Acid) and INH (Isonicotinic Acid Hydrazide). Concentrated specimen or pure cultures are evenly streaked on Bacto-Peizer TB Medium and Sensitivity Disks applied. Zones of inhibition of growth following incubation indicate if an organism is sensitive, moderately sensitive or resistant to the various chemotherapeutic agents.

Peizer TB Medium recommended for sensitivity test procedures is easily and readily prepared from Bacto-Peizer TB Medium Base and Bacto-Peizer TB Medium Enrichment (Peizer and Schecter: Am. J. Clin. Path., 20:682:1950). This is a selective medium suitable for primary culture and simultaneous sensitivity determinations of tubercle bacilli from concentrated specimen.

BACTO-SENSITIVITY TEST MEDIA

Bacto-ATS Medium and Bacto-Lowenstein Medium are available in screw capped tubes with added chemotherapeutic agents. The ATS Medium is prepared according to the formula specified by the American Trudeau Society; the Lowenstein Medium according to the Jensen modification. These media are processed under carefully controlled conditions to give rapid optimal growth of the tubercle bacilli.

Bacto-ATS Sensitivity Media and Bacto-Lowenstein Sensitivity Media are supplied in sets of 12 tubes consisting of 2 tubes of control medium without therapeutic agent and one tube each of the following:

Streptomycin 10 mcg./ml.	INH 5 mcg./ml.
Streptomycin 100 mcg./ml.	Streptomycin 10 mcg. and PAS 100 mcg./ml.
Viomycin 10 mcg./ml.	Streptomycin 100 mcg. and PAS 100 mcg./ml.
Viomycin 100 mcg./ml.	Streptomycin 10 mcg. and INH 1 mcg./ml.
INH 1 mcg./ml.	Streptomycin 100 mcg. and INH 5 mcg./ml.

The above media are also supplied in packages of 12 tubes of any one medium. In addition, for more detailed determinations, ATS Sensitivity Media and Lowenstein Sensitivity Media containing Streptomycin 250 mcg., Viomycin 250 mcg., and INH 0.2 mcg. are available in packages of 12 tubes.

These media are supplied in incubator packages which permit preferred horizontal incubation.

Bulletin No. 141 describing Sensitivity Testing of Tubercle Bacilli is available upon request.

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POSITIONS AVAILABLE

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STAFF PHYSICIAN: A modern, well equipped and staffed, 210 bed tuberculosis hospital located in an attractive mid-western city of 180,000. Applicant must be sober, industrious, and qualified for Michigan license. Salary commensurate with qualifications and experience. Box 141, American Review of Tuberculosis and Pulmonary Diseases, 280 Madison Avenue, New York 16, N. Y.

WANTED: ASSISTANT MEDICAL DIRECTOR, 100 bed tuberculosis hospital, North American Graduate, salary $8500, complete maintenance, apply Medical Director & Superintendent, District Five Tuberculosis Hospital, London, Kentucky, or State Tuberculosis Hospital Commission, New State Office Building, Frankfort, Kentucky.

WANTED: STAFF PHYSICIAN: 215 bed state sanatorium in east central state; previous experience in tuberculosis desirable but not essential, new construction, apartment and utilities furnished, salary $6,000 with increments. Box 142, American Review of Tuberculosis and Pulmonary Diseases, 280 Madison Avenue, New York 16, N. Y.

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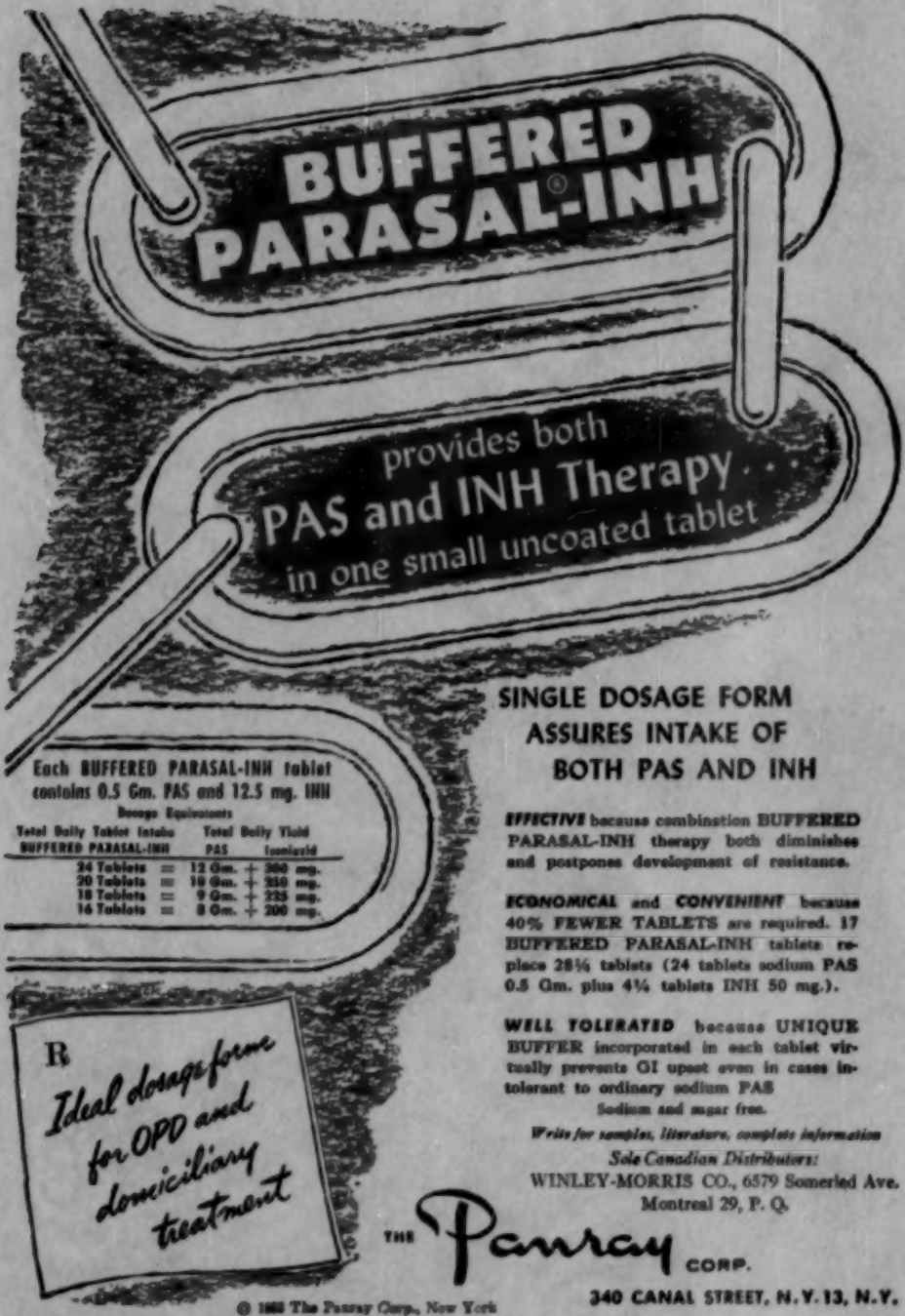

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Total Daily Tablet Intake BUFFERED PARASAL-INH		Total Daily Yield PAS	+ Isoniazid
24 Tablets	=	12 Gm.	+ 300 mg.
20 Tablets	=	10 Gm.	+ 250 mg.
18 Tablets	=	9 Gm.	+ 225 mg.
16 Tablets	=	8 Gm.	+ 200 mg.

R
Ideal dosage forms for OPD and domiciliary treatment

SINGLE DOSAGE FORM ASSURES INTAKE OF BOTH PAS AND INH

EFFECTIVE because combination **BUFFERED PARASAL-INH** therapy both diminishes and postpones development of resistance.

ECONOMICAL and ***CONVENIENT*** because **40% FEWER TABLETS** are required. 17 **BUFFERED PARASAL-INH** tablets replace 28⅓ tablets (24 tablets sodium PAS 0.5 Gm. plus 4¼ tablets INH 50 mg.).

WELL TOLERATED because **UNIQUE BUFFER** incorporated in each tablet virtually prevents GI upset even in cases intolerant to ordinary sodium PAS

Sodium and sugar free.

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